THE MEDICAL JOURNAL OF AUSTRALIA

VOL. II.—15TH YEAR. SYDNEY, SATURDAY, JULY 14, 1928. No. 2.

Authors of articles submitted for publication are requested to read the following instructions and to comply with them.

All articles must be typed with double or treble spacing. Carbon copies should not be sent. Abbreviations should be avoided, especially those of a technical character at times employed in ward notes. Words and sentences should not be underlined or typed in capitals. The selection of the correct type is undertaken by the Editors. When illustrations are required, good photographic prints on glossy gaslight papers should be submitted. Each print should be enclosed in a sheet of paper. On this sheet of paper the number of the figure and the legend to appear below the print should be typed or legibly written. On no account should any mark be made on the back of the photographic print. If no good print is available, negatives may be submitted. Line drawings, graphs, charts and the like should be drawn on thick, white paper in India ink by a person accustomed to draw for reproduction. The drawings should be large and boldly executed and all figures, lettering and symbols should be of suwcient strength and size to remain clear after reduction. Skiagrams can be reproduced satisfactorily only if good prints or negatives are available. The reproduction of all illustrations but especially of skiagrams entails the sacrifice of time and energy and is expensive. Authors are expected to take a corresponding amount of trouble on the preparation of their illustrations, whether skiagrams, photographs, wash drawings or line drawings. The references to articles and books quoted must be accurate and should be compiled according to the following scheme. The order should correspond to the order of appearance in the article. The initials and surnames of the authors, the full title of the article or book, the full (unabbreviated) title of the journal in which the article appears, the date of the issue (day, month and year) and the number of the first page should be given in this sequence.

Table of Contents

[The Whole of the Literary Matter in THE MEDICAL JOURNAL OF AUSTRALIA is Copyright.]

THE FATALITIES AT BUNDABERG— PAGE.

The Report of the Royal Commission—*Continued.*

Appendices—

15. Warning notice and covering memorandum addressed to Dr. J. S. C. Elkington from Dr. F. G. Morgan .. 38
16. Examination of unopened bottles of Batch 86 and of other batches without antiseptic .. 38
17. Results of investigation of partly used rubber-capped bottles of Batch 86 with their previous history .. 38
18. Examination of the remainder of the toxin-antitoxin mixture in the bottle of Batch 86 from which Dr. Thomson made his injections on January 17, 20, 21, 24 and 27 .. 41
19. Examination of the Bundaberg bottle of toxin-antitoxin mixture for iodine .. 43
20. On the possibility of tetanus being the cause of the fatalities .. 44
21. Is it possible that organisms other than staphylococci were present in the Bundaberg bottle of toxin-antitoxin mixture which had died out or been destroyed by the proteolytic activity of the staphylococci? .. 44
22. Report of serological and other characteristics of staphylococci associated with the Bundaberg investigation .. 45
23. The pathogenicity of the Bundaberg staphylococcus to laboratory animals .. 46
24. The production of a skin-reacting toxin from the Bundaberg staphylococcus .. 47
25. The effect of the Bundaberg staphylococcus on toxin and antitoxin separately .. 48
26. On the cultivation of staphylococci in toxin-antitoxin, on the possibility of dissociation of toxin by this method and on the pathogenicity of staphylococci grown in this medium .. 50
27. On the development of turbidity in toxin-antitoxin mixture in relation to the number of staphylococci in Dr. Thomson's bottle on January 27, 1928 .. 60

Appendices—*Continued.* PAGE.

28. Investigation of the content of diphtheria antitoxin in the sera of the survivors of the injection of toxin-antitoxin mixture on January 27, at Bundaberg .. 61
29. On the effect of subcutaneous injection of diphtheria toxin together with the Bundaberg staphylococcus .. 62
30. On generalized staphylococcal infections .. 63

LEADING ARTICLES—

Toxin-Antitoxin Immunization .. 53

CURRENT COMMENT—

Nephritis .. 54

ABSTRACTS FROM CURRENT MEDICAL LITERATURE—

Physiology .. 56
Biological Chemistry .. 57

BRITISH MEDICAL ASSOCIATION NEWS—

Scientific .. 65
Nominations and Elections .. 67

OBITUARY—

John Daniel King Scott .. 67
Thomas Morgan Martin .. 67

CORRESPONDENCE—

Anatomical Abnormalities .. 68
Diathermy Applied to Medicine .. 69
The Chair of Obstetrics and the Appointment of Professors .. 69

POST-GRADUATE WORK—

Post-graduate Work in Melbourne .. 69

BOOKS RECEIVED .. 69

DIARY FOR THE MONTH .. 70

MEDICAL APPOINTMENTS .. 70

MEDICAL APPOINTMENTS VACANT, ETC. .. 70

MEDICAL APPOINTMENTS: IMPORTANT NOTICE 70

EDITORIAL NOTICES .. 70

The Fatalities at Bundaberg.

THE REPORT OF THE ROYAL COMMISSION

(*Continued from page 30.*)

The Commission had of this particular batch for investigation 26 unopened rubber-capped containers and 52 unopened sealed glass ampoules. In addition they received 30 partly used rubber-capped containers and one partly used ampoule (Derham). The partly used ampoule received from Dr. Jackson, Tasmania, could not fairly be investigated, as it had been transferred for postage to an old sterile rubber-capped bottle. The history of these rubber-capped bottles and of several others of Batch 78 (which also contained no antiseptic) is of peculiar interest in view of what happened at Bundaberg. They are dealt with in detail in Appendix 17.

Appendix 15 contains the warning notice and the accompanying memorandum issued by Dr. F. G. Morgan, Director of the Laboratories Division.

APPENDIX 16.

Examination of Unopened Bottles of Batch 86 and of Other Batches Without Antiseptic.

After the accident at Bundaberg, Dr. Morgan (Director Division of Laboratories, Commonwealth Department of Health) at once had six samples of Batch 86 tested by culture into broth and into alkaline meat media. The cultures examined after one, two, three, four, five, seven and thirty days showed no growth and the control tests of the media were satisfactory. At the same time two doses of one and five c.cm. were injected from each ampoule into two guinea pigs of 250 grammes weight. The animals which received one c.cm. showed only loss of weight for 24 hours except one, in which body weight had fallen to 234 grammes on the first day, stayed above 250 grammes to the fourth day, had fallen to 244 grammes by the seventh day and was still below 250 grammes by the thirtieth day, when it had developed a slight paralysis, dragging the hind legs. The animal inoculated with five c.cm. from the same bottle had regained his original weight by the seventh day, weighed 328 grammes on the thirtieth day and was apparently normal. The other five animals inoculated with the larger dose had recovered their original weight between the second and fifth days and none showed any symptoms, having body weights of 340, 354, 380, 352 and 364 grammes on the thirtieth day.

After withdrawing from stock in the presence of witnesses twelve ampoules of Batch 86, Dr. Morgan had the remainder sealed up and stored till handed over to the Commission. Of these 52 ampoules we immediately tested six taken at random (M1, M15, M25, M28, M34 and M40) both for sterility and by injection of five and one c.cm. subcutaneously into guinea pigs of 250 grammes.

These animals were not weighed daily during the first few days, but showed no infiltration. They developed no symptoms, and after twenty days the body weights of those who had received five c.cm. were 307, 345, 327, 337, 324 and 345 grammes respectively. The corresponding animals which had received one c.cm. weighed 332, 314, 327, 320, 288 and 365 grammes. They all appeared then to be in excellent condition and there was no sign of any paralysis.

The sterility tests made into broth and alkaline meat media were also quite satisfactory.

Later we tested a further three ampoules, injecting them in doses of five c.cm. and two c.cm. into guinea pigs. The results on body weight are shown in Table.

None of these animals had any infiltration at the site of injection for the first or second days and in none of them was there any trace of paralysis evident up to the fortieth day.

A number of other ampoules and rubber-capped bottles was tested for sterility and also by injection into guinea pigs prior to inoculating them with the Bundaberg staphylococcus and with other organisms. The following were so tested:

Ampoule M46.—Sterile, injected into a guinea pig in a dose of 1·0 c.cm. No infiltration or other symptoms. Weight on the twentieth day 300 grammes.

Murgon Shire Council, Three Rubber-capped Bottles.—Sterile; 0·9 c.cm. injected into a guinea pig of 250 grammes. Weight on twentieth day 303 grammes. No symptoms.

Brisbane Office Supply 8 (Rubber-capped Bottle).—Sterile; 1·0 c.cm. injected into a guinea pig of 250 grammes. Weight on twenty-first day 313 grammes. No symptoms.

Brisbane Office Supply 10 (Rubber-capped Bottle).—Sterile; 1·0 c.cm. injected into a guinea pig of 250 grammes. Weight on twenty-first day 317 grammes. No symptoms.

Brisbane Office Supply 13 (Rubber-capped Bottle).—Sterile; 1·0 c.cm. injected into a guinea pig of 250 grammes. Weight on twenty-first day 395 grammes. No symptoms.

Brisbane Office Supply 2 (Rubber-capped Bottle).—Sterile; 1·0 c.cm. injected into a guinea pig. Weight on twenty-first day 297 grammes. No symptoms.

Brisbane Office Supply 11 (Rubber-capped Bottle).—Sterile; 1·0 c.cm. injected into a guinea pig. No symptoms. Weight on twenty-second day 363 grammes.

Ten ampoules and six rubber-capped bottles of Batch 86 were, therefore, tested by us for sterility in broth, on blood agar and in alkaline meat media which had been shown to grow *B. chauvei* with ease. Various doses injected into guinea pigs demonstrated that the mixture was over-neutralized and the same fact was attested by the intradermal injection of 0·2 c.cm. from a number of other ampoules and bottles of the same batch during the course of our experiments. All the unopened bottles and ampoules were perfectly clear and remained so.

In none of the unopened ampoules or rubber-capped bottles of this or any other batch of toxin-antitoxin was there any trace of turbidity. All the samples tested by us were found to be sterile. The animal experiments show that the toxin-antitoxin was, as it purported to be, an over-neutralized mixture and our bacteriological tests lead us to the conclusion that the mixture as put on the market is properly safeguarded in regard to the possibility of contamination until the ampoules are opened or the rubber cap pierced for the removal of doses.

APPENDIX 17.

Results of Investigation of Partly Used Rubber-capped Bottles of Batch 86, with Their Previous History.

The results of the investigation of these bottles are shown in the table (see page 40). Except in two cases one or two c.cm. or more of the material was injected subcutaneously into a normal guinea pig of about 250 grammes weight. In many cases the amount available was too small for this purpose. Owing to our early departure for Bundaberg and the demands thrown on the Laboratory Staff at the Walter and Eliza Hall Institute, we were not able to arrange for the daily weighing of all the animals under experiment. Any decrease in weight which may have occurred during the first two or three days after inoculation was, therefore, missed in many cases.

	Original Weight.	Second Day.	Fifth Day.	Eighth Day.	Tenth Day.	Fourteenth Day.	Twenty-third Day.	Thirty-fourth Day.
M29: 5 c.cm.	265	258	266	287	307	335	364	380
2 c.cm.	247	242	257	273	317	326	349	399
M38: 5 c.cm.	260	226	267	270	296	324	369	411
2 c.cm.	244	232	267	259	280	288	327	351
M51: 5 c.cm.	249	222	259	267	300	311	324	383
2 c.cm.	235	215	244	264	298	308	335	371

Cultures were made on to blood agar slopes, into alkaline meat broth and sometimes into glucose brain broth as well. When only a trace of material was left in the bottle, its contents were washed out with sterile broth and the media were inoculated with the washings.

Dr. Randall, of Murgon, used part of one rubber-capped bottle of Batch 86 on one occasion only, his practice being to use materials of that kind only once and to discard any product that had been open for more than an hour. Using Batch 78 and Batch 86, he had inoculated 166 children, three injections being given to each at seven or eight days' interval. Most of the material used was from ampoules of Batch 78 and all of it was devoid of antiseptic. No ill effects followed any of the inoculations. From his single rubber-capped phial, Dr. Randall with some difficulty withdrew about four c.cm. without injecting air. He sterilized the top of the bottle first with spirits and then with iodine.

The following points concerning the technique are taken from a letter to Dr. Elkington in the official file of the Health Department, put in evidence by Dr. Cumpston.

He used six 1·0 c.cm. syringes and 36 needles kept in spirit when not in use. Each needle was used for one injection only and was then cleaned and sterilized by drawing spirit through it several times before replacing it in spirit. The needles were lifted out of the spirit and placed on the syringe with forceps. As soon as ampoules were opened their contents were at once transferred to the syringes. Dr. Randall had not received the "warning notice." His bottle was faintly turbid but sterile on culture.

Dr. Williams, of Brisbane, used a rubber-capped bottle of Batch 86, making injections into two children on November 12, November 19 and November 26, 1927. On November 17 three other children received each 0·5 c.cm. On January 18 and 25 he gave injections to one child and on January 29 a first injection to another child was given. This bottle was, therefore, used repeatedly between November 12, 1927, and January 29, 1928. He had had one small local, but no general reactions following his injections. The remaining contents of his bottle were clear and sterile when we received it. Dr. Williams used methylated spirit as an antiseptic both for his needles and for sterilizing the top of the bottle. It was not his practice to inject air into the bottle in order to remove material from it. He was not aware that Batch 86 contained no antiseptic and had not seen a copy of the warning notice, having bought the material from an agent and not directly from the Commonwealth Health Department, Brisbane.

Dr. Green, of Brisbane, also used a rubber-capped bottle of Batch 86, making sixteen inoculations in six children from it between December 14, 1927, and January 25, 1928. He relied on methylated spirits for sterilizing the top of the rubber cap and injected an equivalent amount of air when withdrawing material in his syringe. He washed out the syringe and needles first with "boiling" water and then with methylated spirits. Dr. Green's bottle was infected with a Gram-positive bacillus. The mixture produced no symptoms in a dose of 1 c.cm. injected subcutaneously into a guinea pig of 250 grammes. Dr. Green had had no reactions in any of the children injected. He had asked specifically for a rubber-capped bottle. He had not received the "warning notice," having obtained the bottle through an agent and not directly from the Commonwealth Health Department, Brisbane.

Dr. Reye, of Brisbane, was not called to give evidence before the Commission, but the following facts have been obtained from the official file of the Commonwealth Health Department from his letter to Dr. Elkington, since confirmed by telegram to the Commission. He obtained his bottle of Batch 86 on January 16, 1928, and gave the first injection on that day. He made seven further injections on January 20, 23 and 27. He used acriflavine 1 in 1,000 to sterilize the rubber cap and the children's skin, but the hypodermic syringe and needle were boiled before use. When several injections were given on the same day, the syringe and needle were boiled between each, unless the children were in one family, when only the needle was boiled. The material in his bottle when examined by us was turbid and contained three species of contaminating organisms. It was without effect on guinea pigs in doses of 1·0 and 2·0 c.cm. He also had received his bottle indirectly through an agent.

Dr. McLean, of Brisbane, also used material out of rubber-capped bottles making repeated injections from the same bottle at intervals. He had inoculated two patients on December 4, 8 and 14, 1927, and had noticed one or two local reactions like a positive Schick test. His technique was to clean the top of the rubber cap with ether. The syringe and needle were sterilized with lysol washed out first with methylated spirit and subsequently with "boiling" water. He always injected an equivalent amount of air into the bottle before withdrawing the mixture. He had received his bottle direct from the Commonwealth Health Department, Brisbane, on November 7, 1927. He had received the "warning notice" by post some time in January and had since abandoned the use of rubber-capped bottles. When we received his bottle the remaining contents were found to be sterile and without effect on a guinea pig in a dose of 1·0 c.cm.

Dr. Earnshaw injected three patients from a bottle of Batch 86, giving each two doses at an interval of five days. The top of the rubber cap was sterilized with methylated spirit or iodine and the syringe and needle by boiling. He did not inject air into the bottle to withdraw samples. He had had no ill effects following any injections other than a slight local reaction in the older children. He had purchased his bottle on November 7, 1927, from the Commonwealth Health Department, Brisbane, and had received a copy of the warning notice some time in January, 1928. The remaining contents of his bottle were contaminated with a *Staphylococcus albus*, but in a dose of 1·0 c.cm. the mixture produced no ill effects when injected subcutaneously into a guinea pig.

Dr. Elwell, of Stanthorpe, injected three children from his bottle on three occasions each, December 1, 6 and 13. He sterilized the syringe by boiling, the needles by placing them in methylated spirits for fifteen minutes and the rubber cap by filling the depression with methylated spirits for ten minutes before withdrawing the sample. He used 0·5% phenol as a diluent for the doses. The remaining mixture in Dr. Elwell's bottle was clear and sterile. He had received the bottle about December 1, 1927, directly from the Commonwealth Serum Laboratories, Melbourne. He had not received the "warning notice."

Dr. Meyers used a bottle of Batch 78A to inject two patients, one received three injections on December 9, December 16 and December 21, 1927, and the other, one injection only on January 23, 1928. He had received the bottle, a partly used one, without wrappings, from the Children's Hospital, Brisbane. He was not informed that it contained no antiseptic. The contents of this bottle were perfectly clear when it reached us in Melbourne. Dr. Meyers's practice was to clean the rubber cap with ether, with ether and alcohol or with dilute lysol. The syringe was sterilized in one or more of these liquids

Dr. Patterson, Superintendent of Children's Hospital, Brisbane, had used a number of rubber-capped bottles of Batch 86. Repeated inoculations were made from the same bottle which, since many inoculations were made on one day, did not last more than three or four days. Lysol was used to sterilize the rubber cap and the needles and syringe were sterilized in lysol and subsequently washed out with distilled water.

Dr. Patterson had used active immunization to control institutional diphtheria with apparently good results. In eighteen months prior to the introduction of this measure there were 53 cases of diphtheria presumably contracted in hospital and in the last seven months there had been one only. He had observed four cold abscesses following inoculation among 1,020 children immunized. Two injections of 0·5 c.cm. of toxin-antitoxin mixture were used at five day intervals so as to get rapid immunization.

There were no cases of ill effects resulting from the injections of toxin-antitoxin mixture. The case of one child named Nock was fully investigated by a member of the commission and by Dr. S. F. McDonald, of the honorary staff of the hospital. They were satisfied that the child's condition was not related directly or indirectly to the immunizing injections he had received.

Dr. Patterson had not seen the "warning notice," nor was he aware that the mixture he had been using contained no antiseptic.

Dr. J. V. Duhig, the pathologist in charge of the hospital laboratories, thought that the technique used for injections was so good as to preclude any possibility of serious contamination, particularly as the bottles were used up so rapidly. He had requested the Commonwealth Health Department, Brisbane, to let the hospital have rubber-capped bottles in place of 1·0 c.cm. ampoules which he found wasteful and expensive. He had received a copy of the "warning notice" at this time from the Commonwealth Health Department, Brisbane.

None of the remaining contents of any of the bottles used in this hospital were available for examination by the Commission.

Under the direction of Dr. Tilling, 23 children were inoculated by specially trained nurses from the contents of rubber-capped bottle of Batch 86 between January 3 and 16, 1928. Inoculations were given on January 3, 4, 6, 9, 11, 12, 13 and 16. This bottle was completely used.

Dr. Tilling was unaware that the bottles of this batch contained no antiseptic. These cases were injected at the Children's Hospital. Miss Barstow was one of the nurses who had carried out these injections. She had noted no ill effects following any of the injections. She apparently did not know that the material she was using contained no antiseptic. The technique used was to sterilize the rubber cap and the syringe which was kept for this purpose only, with ether. The needles were sterilized with strong lysol.

Dr. Lowe, of Heidelberg, had given up using rubber-capped bottles since "about two months before Christmas." He had, however, used part of a rubber-capped bottle of 78A (bottled November 28, 1927). He was apparently using toxin-antitoxin mixture for the treatment of various conditions and not for immunization. It was his practice to make repeated injections from the same bottle at varying time intervals. One of his patients, a woman of 48 years of age, became very ill with vomiting and high temperature after an injection. He opened the bottle from which this injection was made and found it contaminated. He attributed this to air contamination in withdrawing samples from the bottle. The batch to which this bottle belonged is uncertain. The bottle itself was not available for examination. He used flavine to sterilize his syringe and needles and methylated spirits to sterilize the rubber cap. Dr. Lowe did not remember any of the notices packed round the bottles inside the cartons.

Dr. A. P. Derham, of Melbourne, had used a sealed glass ampoule of Batch 86. Four c.cm. were used on one day at the end of December and the cut end of the ampoule was sealed with cotton wool moistened with spirits. It was his practice to use the ampoules on the second day as well as the first and even on the third day after opening them, the opened ampoule being in the meanwhile plugged with cotton wool. This ampoule was sterile when examined by the Commission.

RESULT OF EXAMINATION OF PARTLY USED RUBBER-CAPPED BOTTLES OF BATCH 86.

Source of Bottle.	Whether Clear or Turbid.	Cultures.	Animal Inoculations.	Result.
Dr. Thomson, Bundaberg	Turbid	*Staphylococcus aureus*	Numerous, described separately	
Dr. Randall, Murgon	Slightly Turbid	Sterile	Not inoculated	
Dr. Williams, Brisbane	Clear	Sterile	Guinea pig, 1·5 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 295 grammes.
Dr. Green, Brisbane	Turbid	Gram-positive bacillus	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 340 grammes.
Dr. Reye, Brisbane	Turbid	Staphylococcus, Gram-positive bacillus, Gram-negative bacillus	Guinea pigs, 2·0 c.cm. and 1·0 c.cm., subcutaneously	No symptoms. Weights on twenty-first day, 339 grammes and 338 grammes.
Dr. McLean, Brisbane	Clear	Sterile	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 332 grammes.
Dr. Earnshaw, Brisbane	Turbid	*Staphylococcus albus*	Not inoculated	
Dr. A. P. Derham, Melbourne (Ampoule)	Clear	Sterile	Guinea pig, 4·0 c.cm., subcutaneously	No symptoms. Weight on thirtieth day, 435 grammes.
Dr. Elwell, Stanthorpe	Clear	Sterile	Not inoculated	
Dr. Hilda Bull, I, Melbourne	Clear	Sterile	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 340 grammes.
			Rabbit, 1·0 c.cm., subcutaneously	No symptoms by thirty-second day.
Dr. Hilda Bull, II, Melbourne	Clear	Sterile	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms except loss of weight by twenty-first day, which had fallen from 234 grammes to 214 grammes. Weight on thirty-second day, 271 grammes. No paralysis.
Dr. Hilda Bull, III, Melbourne	Faintly Turbid	*Staphylococcus albus*	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 344 grammes.
Dr. J. A. Goldschmid, 989/3 (containing antiseptic)	Clear	Sterile	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-first day, 319 grammes.
Melbourne Health Department, I	Turbid	Gram-positive sporing bacilli, Gram-negative bacilli	Guinea pig, 1·5 cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 340 grammes.
Melbourne Health Department, II	Turbid	Gram-positive tetracoccus	Guinea pig, 2·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 395 grammes.
Melbourne Health Department, III	Clear	Sterile	Guinea pig, 1·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 360 grammes.
Melbourne Health Department, IV	Clear	Sterile	Guinea pig, 2·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 374 grammes.
Melbourne Health Department, V	Clear	Sterile	Guinea pig, 3·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 400 grammes.
Melbourne Health Department, VI	Clear	Sterile	Guinea pig, 0·5 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 358 grammes.
Melbourne Health Department, VII	Clear	Sterile	Guinea pig, 2·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 387 grammes.
Melbourne Health Department, VIII	—	Gram-positive and Gram-negative filamentous bacilli	Not inoculated	
Melbourne Health Department, IX	Clear	Sterile	Guinea pig, 1·0 c.cm., subcutaneously	No symptoms. Weight on twenty-fourth day, 365 grammes.
Melbourne Health Department, X	Turbid	Gram-positive bacilli, Gram-negative bacilli	Not inoculated	
Melbourne Health Department, XIII	Turbid (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XIV	Turbid (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XV	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XVI	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XVII	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XVIII	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XIX	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XX	Clear (only trace)	Sterile	Not inoculated	
Melbourne Health Department, XXI	Clear (only trace)	Sterile	Not inoculated	

Dr. Hilda Bull who was immunizing school children for the Melbourne City Council, had used a large number of rubber-capped bottles of Batch 86 and had been warned previously by Dr. Morgan (Director of the Division of Laboratories, Commonwealth Department of Health) that they contained no antiseptic. She used twenty-four bottles of Batch 86. The top of the rubber cap was sterilized by first pouring on methylated spirit and then burning it off. The three bottles labelled Bull 1, 2 and 3 and the nineteen other partly used bottles (labelled Melbourne Health Department) tested by us, came from this source. Only five out of twenty-two were contaminated and eleven contained only a trace of material so that a large number of inoculations must have been made from each. No risk attached to these contaminations as it was her practice to use the whole contents within one hour and discard material that was left. She had observed no ill effects following a large series of inoculations. Growth in some of the bottles had taken place in the long period which had elapsed since she used them.

The only rubber-capped bottle containing antiseptic which appears in the table is from Dr. J. A. Goldschmid, of Brisbane, and belonged to Batch 989/3. He had inoculated two children on December 1, 8 and 15 from this bottle without untoward results. The remaining contents were sterile when examined by the Commission and in a dose of 1·0 c.cm. produced no ill effects when injected subcutaneously into a guinea pig.

The use of rubber-capped bottles containing toxin-antitoxin with or without antiseptic, when large numbers of children are to be inoculated on one occasion, is a very convenient one and perfectly satisfactory when the rule followed by Dr. Bull and Dr. Randall, of discarding all material not used within an hour, is adhered to. It is evident that the practice of using the same rubber-capped bottle on a number of occasions at widely-spaced intervals is very prevalent, possibly because medical men are accustomed to administer vaccines containing antiseptic in this way.

Of the rubber-capped bottles which the Commission examined, 30% were shown to be contaminated. Considering the large number of injections given from them and the possibilities of air contamination, this percentage is not excessive (Appendix 4), though some of the contaminations are doubtless referable to faulty technique.

We have not examined a series of partly used bottles containing antiseptic, but are assured from other experiments that with antiseptic present (Appendix 4), the percentage of contamination would be negligible.

APPENDIX 18.

Examination of the Remainder of the Toxin-antitoxin Mixture in the Bottle of Batch 86 from which Dr. Thomson made his Injections on January 17, 20, 21, 24 and 27.

Subsequent History of this Bottle.

On the afternoon of January 28, Dr. Thomson handed the bottle to the Mayor in the presence of Mr. McLeod. It was taken by him to Dr. McKeon's surgery where in the presence of Messrs. Marshall, Finnimore, McLeod, McCarthy (the Town Clerk), and the Mayor, Dr. McKeon withdrew about 0·3 c.cm. using a sterile syringe, but without sterilizing the top of the rubber cap.

This portion of the contents was placed in a small sterile rubber-stoppered phial. The original bottle and the phial were now made up in separate packages and sealed by Mr. Marshall in the presence of the above witnesses. The small phial was placed in the safe at the Council Chambers and handed over to Dr. Tilling on January 31. Dr. Tilling took it to Brisbane and placed it in safe deposit, together with another sealed package containing the needles and syringe used by Dr. Thomson. They were forwarded by registered post to the Commission at Melbourne on February 3.

The sealed package containing the original bottle was placed in the safe at the Council Chambers and handed to Dr. Murray on January 30. Dr. Murray took it to Brisbane and handed it to Dr. Elkington who enclosed it in a second sealed package and transmitted it by post to the Health Department, Melbourne. On arrival on February 3, 1928, the registered package was at once handed over to the Commission and was opened at the Hall Institute by the members of the Commission who found that the wrappings placed on the package at Bundaberg and Brisbane had not been tampered with, and that the seals were intact. The second sample arrived by post at the Hall Institute on February 6, 1928, and when opened the seals of the original packages made up in Bundaberg were also found to be intact.

The bottle was examined on the evening of its arrival in Melbourne. It contained 1·45 c.cm. of turbid fluid, 1·25 c.cm. of which was removed, after the rubber cap had been sterilized by methylated spirit and flaming, with a syringe sterilized by boiling in water for twenty-five minutes. The operation of withdrawing the contents was carried out in a dust-free cabinet. One-fifth of a cubic centimetre was subsequently estimated as remaining in the bottle.

The total amount actually used in immunizing injections in Bundaberg was said to be 7·0 c.cm. With 1·47 c.cm. in this bottle and 0·3 c.cm. previously withdrawn by Dr. McKeon forming the second sample [B.T.A.T. (2)] examined, there remains 1·23 c.cm. to be accounted for or possibly slightly more if, as is usual, generous measure is given in filling the bottle. It is not unreasonable to suppose that this is fully accounted for by wastage in the dead space of the syringe and needle and in adjusting dosage when 45 separate injections on five occasions have been given. Dr. Thomson states definitely in his evidence that more was removed on each occasion than was necessary.

Microscopical and Bacteriological Examination of the Fatal Bottle.

A.—Microscopical.

Films were made immediately from the contents of the rubber-capped bottle of toxin-antitoxin used at Bundaberg (labelled B.T.A.T.). When spread on glass slides a distinct acid odour could be recognized with the nose close to the slides. The films were stained by Gram's and Ziehl-Neelsen's methods. They showed numerous Gram-positive cocci which varied somewhat in size and arrangement. They were mostly in pairs or as single cocci, but also in short chains of four to six cocci. There was also much amorphous and granular Gram-negative material. Seven films altogether were examined and in five of them a few clumps of yeast were found, but apart from the staphylococci, no other organisms of any kind.

B.—Bacteriological.

Cultures were made immediately from the aseptically removed contents of the rubber-capped bottle on or in the following media, with the results as shown:

(1) Two rubber-capped bottles of toxin-antitoxin of Batch 78A prepared recently at the Commonwealth Serum Laboratories and containing no antiseptic. One-hundredth of a cubic centimetre was injected through the rubber cap into each bottle. One bottle was incubated at 37° C. and the other left at room temperature (20-32° C.), whilst a third uninoculated bottle was placed in the incubator at 37° C. The latter remained perfectly transparent during the subsequent five days, and at later dates it was still quite clear. The inoculated bottles became slightly turbid on the following day, particularly that at 37° C., and the turbidity of both was greater on the second day. On the third day some of the toxin-antitoxin was removed, films were made and also subcultures into alkaline meat medium and glucose brain broth. The films showed only Gram-positive cocci, diplococci, short chains of three and four cocci, and many small groups or clusters of cocci. Films made later from the subcultures in the above-mentioned media showed a very similar picture and hanging drop preparations showed mainly diplococci, but also short chains of four cocci and clusters.

(2) Four tubes of glucose brain broth. These were inoculated with one platinum loopful, one of each incubated aerobically and anaerobically at 37° C. and at room temperature. The latest pattern of McIntosh and Fildes anaerobic jars was employed for all anaerobic cultivation. Growth was rapid aerobically at 37° and acidity

without gas was noted within 24 hours, whilst the same results were obtained at room temperature only on the second day. On the fifth day the medium became alkaline and on the sixth a small amount of gas was noted. Anaerobically there was more growth at 37° than at room temperature. Films from these cultures showed Gram-positive cocci, diplococci, chains of three and four and even as many as six cocci, also tetrads and small clusters. These aerobic and anaerobic cultures were plated on blood-agar aerobically and anaerobically and the anaerobic brain broths re-inoculated into brain broth. The blood-agar plates showed numerous glistening white colonies resembling at first *Staphylococcus albus*. A slight degree of hæmolysis was slowly produced, and the colour of the colonies became slightly yellowish. There were no very small colonies where the colonies were spaced out.

(3) Four tubes of glucose broth prepared at this Institute were similarly inoculated from the B.T.A.T. and incubated as in (2). Turbidity and later greyish-white deposit were noted in all tubes and plating on blood-agar gave only colonies of staphylococci. One small colony was picked off on to an agar slope, but gave a typical growth of staphylococcus. Films from the glucose broths showed again short chain formation as well as diplococci and clusters.

(4) Four sloped agar tubes (hormone-agar) incubated as in (2). Aerobically at 37° C. there was a good growth in 24 hours. It resembled *Staphylococcus albus* and showed the typical microscopical appearances of the staphylococci. After three days the growth was faintly yellowish, but on keeping never became typically *aureus*. At room temperature growth was decidedly slower. Anaerobically there was a good whitish growth at 37° C. in three days, no visible growth in three days at room temperature, but a whitish growth appeared later. Examination of films showed only staphylococci.

(5) Agar plates. One platinum loopful of B.T.A.T. inoculated into melted agar, well mixed and plated (shake cultures) gave innumerable colonies. One loopful of one in ten dilution of B.T.A.T. gave a moderate number of colonies, of one in a hundred dilution a few colonies only, of one in a thousand dilution very few colonies. At the time no counting of colonies was carried out, but experience obtained since by repeating this experiment with other bottles allows us to be reasonably certain that the number of living cocci in the bottle when received by us on February 3 was not greater than 200 millions per cubic centimetre. The whitish surface colonies grew to a considerable size and became of a slight yellowish colour in three days. The deep colonies were lenticular. Five of the smaller surface colonies were picked off on to sloped agar, but proved to be of the same type. Anaerobic plates were also made in the same way with similar results. All films showed only staphylococci.

(6) Glucose-agar plates. The glucose agar was prepared at the Commonwealth Serum Laboratories. Shake cultures were made as in (5) and incubated aerobically and anaerobically. The results were much the same as in (5).

(7) Blood-agar plates. Surface stroke cultures were made, using two plates each for undiluted B.T.A.T. and for dilutions of one in ten, one in a hundred and one in a thousand. One set was incubated aerobically and the other anaerobically. On the aerobic plates falling numbers of colonies resembling *Staphylococcus albus* were found next day. After three days there was slight hæmolysis. Nine colonies were picked off and subcultured. Staphylococci were alone found in films of these subcultures. There was a good growth also on the anaerobic plates and seven of the smaller colonies were subcultured on to sloped blood-agar and incubated anaerobically. In 24 hours these showed whitish confluent growth or separate colonies, but films of these showed staphylococci, mostly clusters, but also diplococci and chains of three or four cocci.

(8) Sloped serum-agar prepared at the Commonwealth Serum Laboratories. Stroke cultures were made with one loopful of one in a hundred and one in a thousand dilutions of B.T.A.T. were made and incubated aerobically and anaerobically. The lesser dilution gave whitish discrete colonies and some confluence, the greater dilutions only discrete colonies. Two colonies of smaller type were subcultured but proved to be staphylococci. After three days the confluent growth was slightly yellowish. The anaerobic cultures gave good growths of staphylococci within three days.

(9) Glucose broth prepared at the Commonwealth Serum Laboratories. The results were much the same as in (3) and do not warrant separate description.

(10) Plain broth. Aerobic cultivation at 37° C. gave a moderate turbidity with some deposit in twenty hours, and on the third day there was a scum on the surface against the glass of the test tube. Plating on blood-agar gave only staphylococci which produced hæmolysis slowly. Anaerobic cultures gave only staphylococci.

(11) Alkaline meat medium prepared at the Commonwealth Serum Laboratories. One set was covered with a layer of paraffin and the other was not covered. Aerobic cultivation at 37° C. gave turbidity in 24 hours. Plating on blood agar gave only staphylococci. No gas was observed in subsequent observations up to twenty days. Films of the cultures at room temperature showed only staphylococci. Anaerobic cultivation gave only staphylococci and there was no gas production.

Some of the one in ten dilution of B.T.A.T. was heated at 60° C. for 30 minutes and then incubated on to serum-agar and into glucose-brain broth. No growth took place within ten days.

To exclude the possibility that our media were unsuitable for the growth of anaerobic and sporing forms we inoculated control tubes with *B. chauvei*. Good growth was obtained with all the media tested.

Examination of Sample Withdrawn by Dr. McKeon.—The remainder of the contents of the fatal bottle was received in a small ampoule sealed with a small rubber cork on February 7. It was labelled by us B.T.A.T. 2. As described elsewhere, the volume of the contents was very small. Indicators showed the pH to be between 7·5 and 8. Films indicated a smaller number of organisms than in the original bottle. They were Gram-positive cocci, most numerous as single cocci, but also many diplococci and less often three cocci in a row or forming a triangle. No definite chains or bacilli were seen. There was much Gram-negative material, mostly in coccoid forms; a few doubtful forms which were probably yeasts were present.

The contents were plated on blood-agar. Most of the colonies were white resembling those grown from the original bottle, and there were a few smaller colonies. Subcultures of these large and small colonies were made on sloped blood-agar. One large and one small colony gave a confluent whitish growth of staphylococci resembling that found in the original bottle, but one small colony gave an orange-yellow confluent growth of staphylococci. It was not known whether this latter was another contamination made in Bundaberg in withdrawing the sample from the bottle to the ampoule, a varient from the less orange type, or an air contamination of the blood-agar plates.

Cultures were also made in two alkaline meat tubes and in two glucose-brain broths, incubated aerobically. After two days the former were turbid and one showed a bubble or two of gas. Films, however, showed only Gram-positive cocci in short chains and in groups. No bacilli could be found.

The glucose-brain broths were turbid and showed acidity in two days. Films showed cocci, mostly in clusters. No bacilli could be found.

No animal inoculations were made direct from this second sample.

Animal Inoculations.

There was obviously an insufficient amount of material available in the original bottle for its complete characterization as a mixture of diphtheria toxin and antitoxin by subcutaneous inoculation into guinea pigs and, for reasons detailed elsewhere in this report, it seemed unlikely that excess of diphtheria toxin would prove to be responsible for the tragedy.

At a later stage on our return from Bundaberg, we used the intradermic test with and without added antitoxin to determine the presence of free toxin in some of this original material.

This was done by injecting into the skin of a white guinea pig in a total volume of 0·2 c.cm. the following doses of the material: 0·02 c.cm., 0·01 c.cm., 0·005 c.cm.

and 0·002 c.cm. with and without 0·002 c.cm. of standard antitoxin. None of these doses produced the slightest skin reaction, and as from $^1/_{250}$ to $^1/_{500}$ of the M.L.D. of diphtheria toxin gives a definite skin reaction, it may fairly be concluded that at the time when these tests were performed, nearly a month after the tragedy, the material did not contain a significant amount of free diphtheria toxin. Our subsequent experimental evidence has shown the remarkable stability of old diphtheria toxin in the presence of the staphylococcus which was isolated from the material (Appendix 25) and it may, therefore, be regarded as unlikely that significant excess of diphtheria toxin was present at any time in the bottle.

In the immediate investigation of the material for the presence of some unknown toxin of extreme potency or of a virulent living organism, we considered it advisable to test its action in as many species as possible and to utilize intravenous injections which would afford small doses of any toxic substance present the best chance of producing fatal effects.

The animals which we used were rabbits, guinea pigs and mice. In addition to intravenous injections in these animals, we decided to give to three monkeys doses comparable with those administered to the children at Bundaberg. The monkeys were said to be about three years old and weighed 4, 3·5 and 2·6 kilograms respectively. One-fifth of a cubic centimetre, 0·1 c.cm. and 0·2 c.cm. of the contents of the fatal bottle were given subcutaneously. The first of these animals was protected against any possible free diphtheria toxin by the subcutaneous injection three hours earlier of 6,000 units of antitoxin. None of them had any illness following the injections. Though many living organisms were shown to be present in the injected material, no abscess resulted at the site of injection. The animals were in excellent condition two months later and were used again for the experiments detailed in Appendix 26.

Rabbits.

Two rabbits of 2·4 and 1·8 kilograms were inoculated intravenously each with 0·1 c.cm. of the material. Both of these became definitely ill within twenty-four hours. One died on the fifth day with a broncho-pnuemonia and septic infarcts in both kidneys from which a pure culture of a staphylococcus was obtained (identical with that isolated from the original material). The second was killed on the thirtieth day when moribund. It developed a stiff and painful left elbow joint forty-eight hours after injection and appeared to be definitely ill for some days. On the twenty-sixth day complete paralysis of both hind limbs developed, as has been described in experimental staphylococcal infections in this animal. At autopsy there were no obvious lesions in any of the joints and the brain and spinal cord were macroscopically normal. The only definite macroscopic lesions were extensive organized infarctions of both kidneys, which were sterile on culture.

Guinea Pigs.

Three guinea pigs of about 250 grammes weight were injected, one with 0·05 c.cm. of the material subcutaneously, and two with 0·03 and 0·035 c.cm. intravenously. A fourth guinea pig received 0·02 c.cm. of the material which had been heated to 60° C. for half an hour. The subcutaneous injection caused no trace of infiltration. The other animals which received intravenous injections, were not very well for a few days and showed some loss of weight. They all survived and three of them when killed and examined nearly a month later showed no obvious lesions at autopsy. The fourth animal received an intravenous injection of 1·0 c.cm. horse serum on the fifty-fourth day when it weighed 400 grammes and no anaphylactic symptoms resulted.

Mice.

Eight mice weighing from 16 to 26 grammes were injected intravenously by the tail vein with 0·1 c.cm. of a 1 in 10 dilution of the material. They showed no symptoms of illness following the injection except one weighing 16 grammes in which part of the injection was given subcutaneously. This animal was very sick within twenty-four hours, and was found dead on the second day. No definite lesions were present at autopsy. A second mouse was found dead on the fifteenth day and the uterus was distended with caseous material.

Two mice of 17 and 15 grammes were injected intravenously by the tail vein with 0·01 c.cm. of the material diluted one in ten with saline and heated to 60° C. for half an hour. These also showed no ill-effects following the injection.

The results of these inoculations into animals are very unimpressive. There is nothing to suggest the presence of free diphtheria toxin in the bottle and its contents show in the dosage used no great toxicity or pathogenicity for laboratory animals. It seems probable that we are concerned either with great loss of activity of the contents of the bottle during the week which elapsed between its use on January 27 and its examination by us on February 3 or with an activity which could only be manifested by injection into young susceptible human subjects. We are inclined to think that the material had lost some of its activity, that the doses used would with advantage have been larger and administered to fewer animals, but that it is not unlikely that all the laboratory animals used were unsuitable for the purpose of testing its activity.

Subculture from B.T.A.T. 2 into a bottle of toxin-antitoxin mixture (M.S.C. 3.) incubated at room temperature grew rapidly. Guinea pigs inoculated on several successive days showed no symptoms and smears of the material contained only cocci. When, however, the residual contents of the bottle were spun down and the deposit examined, two small clumps of yeast were found. No yeasts were found on any of the cultures of B.T.A.T. on solid media and later attempts to grow them on Sabouraud's media were unsuccessful.

The only organisms which could be recovered from the original bottle and the sample removed by Dr. McKeon were staphylococci. The yeasts found in the original smears were not recovered by culture and were very probably an air contamination. It is extremely unlikely that they had any pathogenic activity.

APPENDIX 19.

Examination of the Bundaberg Bottle of Toxin-Antitoxin for Iodine.

We were interested in this question from the general standpoint of the possibility of the entry into the rubber-capped bottles of liquids used for sterilizing the top of the rubber cap. Iodine as used by Dr. Thomson lends itself particularly well to the investigation of this point. Other liquids used for the same purpose might quite conceivably have some deleterious action on biological products *e.g.* pyridine present in methylated spirit. Apart from these considerations it seemed possible that iodine might have played some part in the accident we were investigating.

When we had withdrawn as much as possible of the remaining contents of the bottle, we asked Mr. F. H. Holden, the Research Biochemist of the Walter and Eliza Hall Institute, to estimate the amount of iodine present in the remaining contents. These contents were estimated to be 0·075 c.cm. by washing out and weighing after removing the rubber cap. The method used for iodine estimation was capable in practised hands of detecting with certainty 0·0005 mgm. The reagents were free from iodine in quantities capable of detection by the method used. 0·003 mgm. of iodine were found corresponding to 0·04 mgm. per c.cm. of toxin-antitoxin mixture.

This of course represents the maximal amount of iodine ever present as it was doubtless added to by successive needle punctures.

We were not able to show that iodine in half this concentration made possible any appreciable difference to the growth of organisms or produced any product toxic to laboratory animals. Neither did it aid in the dissociation of toxin from antitoxin. Toxin was quite stable in its presence in this concentration. In higher concentrations it inhibited the growth of organisms without producing any toxic products in toxin-antitoxin mixture.

The presence of iodine in the bottle is also suggestive of the ease with which organisms could be carried in through the puncture in the rubber cap if the surface of the latter were not efficiently sterilized.

APPENDIX 20.

On the Possibility of Tetanus Being the Cause of the Fatalities.

We were not impressed in this connexion by evidence obtainable as to the symptomatology presented by the children at Bundaberg nor did any local practitioner who saw the affected children, make a diagnosis of tetanus. The microscopical and cultural examination of the bottle of toxin-antitoxin mixture used by Dr. Thomson lent no support to this view, nor were we aware of such great individual variations in susceptibilty to tetanus toxin as was shown by these children to the inoculated material. As shown elsewhere, several children died within twenty-four hours and several showed no symptoms following an injection of 0·125 c.cm., and moreover, none of the children inoculated three and six days previously suffered any illness.

The two questions we endeavoured to answer were (i) Would the addition of a small number of tetanus bacilli to toxin-antitoxin result in the production of tetanus toxin in doses possibly lethal to children? (ii) Would tetanus bacilli or their spores, having grown in the toxin-antitoxin, be demonstrable after a lapse of time or might the bacilli multiply, produce toxin and then disappear?

An agar culture of *B. tetani* (*Clostridium tetani*) was obtained from the Commonwealth Serum Laboratories. Films showed mostly typical drum stick forms, but also a few free spores, non-spored forms and long forms. A weak emulsion was made in normal saline and 0·1 c.cm. injected into one rubber-capped bottle (referred to later as No. 1), of toxin-antitoxin containing no phenol (Batch 86), into another (No 2) containing 0·5 per cent. phenol (batch 111), also into two bottles (Nos. 3, batch 86 and 4, batch 111) which received in addition 0·2 c.cm. of a 1 in 10,000 dilution of an emulsion of the Bundaberg staphylococcus, an inoculation of 1,000 to 2,000 cocci per c.cm. of toxin-antitoxin in the bottle. These four bottles were placed in an incubator which was at first set at 75° F. (24° C.) and later at 80° F. (26·5° C.).

Guinea pigs were inoculated subcutaneously with 0·2 c.cm. from each of these bottles after two days, with 0·5 c.cm. of Bottles 3 and 4 after five days, with 0·5 c.cm. of each bottle after eight days, with 0·05 c.cm. of Bottle 1 after ten days, with 0·5 c.cm. of each bottle after thirteen days and after twenty days. None of these animals showed any local lesions or any signs of tetanus during the 34 days following the commencement of these inoculations. Altogether, therefore, nineteen animals were inoculated at various intervals and all survived. Their average weight at the time of inoculation was 231 grammes. A few showed a slight loss of or failure to gain weight for a few days, but during the period of observation stated above the average gain in weight was 73 grammes. There were no deaths among these animals, which remained in splendid condition, during a further period of three weeks, when they were discarded.

When the bottles were inoculated, subcultures were also made from the same agar culture of *B. tetani* on to agar and into alkaline meat medium. These were incubated at 37° C., the former in an anaeobic jar and good growths were obtained which appeared to be pure when examined microscopically. Subcultures on agar incubated aerobically showed no growth. One fifth of a cubic centimetre of a three days' culture in alkaline meat medium killed a guinea pig in two days. No infiltration could be felt at the site of inoculation. Also a guinea pig inoculated with 0·2 c.cm. of a four days' culture in plain broth was found in convulsions nineteen hours later and died after a further two and a half hours.

Subcultures were made from the bottles of toxin-antitoxin at intervals. No 3 bottle became slightly turbid in two days, Nos. 1, 2 and 4 were still perfectly clear after six days. The turbidity in No 3 bottle was due mainly to the staphylococcus as was shown by plating on agar anaerobically. Plating after two days gave no tetanus colonies, after five days a few filamentous forms were found amongst the staphylococci in films made from the plate, and after ten days subcultures from Bottle No. 3 into alkaline meat medium gave gas formation and both *B. tetani* and staphylococci were found microscopically, whereas a meat tube heated to 80° C. for fifteen minutes after inoculation gave only *B. tetani*. Plating from this bottle after twelve days gave a mixture of staphylococci and *B. tetani*. Films made from a drop of the contents showed a few spored and unspored bacilli and a few gram-positive diplococci and cocci. Some of this bottle was also centrifuged and the deposit showed also *B. tetani* and staphylococci. In fact there appeared to have been some growth of *B. tetani* in No. 3 bottle, though 0·5 c.cm. did not cause any ill-effects in the guinea pig.

Subcultures into meat media from Bottles 1, 2 and 4 also gave prompt growth of *B. tetani*, though films made from the toxin-antitoxin and from the deposit obtained by centrifuging showed few bacilli. Finally, Bottle No. 3 (containing no phenol and inoculated with both *B. tetani* and the Bundaberg staphylococcus) was subcultured on the twenty-first day into a tube of meat medium and this was immediately heated to 80° C. for fifteen minutes. After several days' incubation at 37° C., 0·1 c.cm. of this subculture killed a guinea pig in 43 hours, death being preceded by convulsions, whilst 0·2 c.cm. killed in 21½ hours.

We are of the opinion that the contamination by the Bundaberg staphylococcus took place at Bundaberg during inoculations on some day previous to January 27. It seems reasonable to suppose that a contamination by *B. tetani* would have occurred either at Bundaberg or at the Commonwealth Serum Laboratories. These experiments do not lend any support to a contamination by *B. tetani* either at the Commonwealth Serum Laboratories or at Bundaberg. With regard to the Commonwealth Serum Laboratories there is no evidence before us that any other bottle of Batch 86 or of 78A gave rise to tetanus, though large numbers of inoculations have been carried out with them. This contingency would mean that first of all the toxin-antitoxin was contaminated by *B. tetani* at the laboratories in November, that the bacillus began to multiply subsequently,[1] that the bottle was then contaminated at Bundaberg by the staphylococcus, that a lethal dose of tetanus toxin was produced only between January 24 and 27 so that on January 20 and 24 for example, numbers of children could be inoculated without the slighest suggestion of tetanus toxin poisoning, and finally, that *B. tetani* disappeared so completely by February 3 that they could neither be found in films nor cultivated in suitable media.

Now in the one bottle of Batch 86 (Bottle No. 1) which received a small inoculation of *B. tetani* only, there appeared to be little if any multiplication or toxin production in twenty days, though *B. tetani* could be cultivated from the bottle by inoculating small quantities into meat medium or by plating on agar. The growth of the staphylococcus appeared to favour a slight and slow growth of *B. tetani*, but this was scarely appreciable within eight days, and there was no evidence of the production of tetanus toxin in doses lethal to guinea pigs. It was not thought necessary to look for the presence of doses smaller than this.

APPENDIX 21.

Is it Possible that Organisms other than Staphylococci were present in the Bundaberg Bottle of Toxin-Antitoxin Mixture which had died out or been destroyed by Proteolytic Activity of the Staphylococci?

The examination of smears of the material in the bottle of Batch 86 received from Dr. Thomson revealed the presence, in addition to enormous numbers of cocci, of a few clumps of yeasts which could not be recovered by culture. Their presence and the results of our experiments on air contamination, raised the question as to whether it

[1] With the exception of the time taken by transport to Brisbane this bottle was kept at low temperatures until January 6. From this date to January 27 it was kept at room temperatures averaging not higher than 80° Fahrenheit (26·5° C.).

was possible that some other organism had once been numerous and had first been overgrown and subsequently dissolved by the activity of the staphylococci which alone were cultivated from the bottle in a large number of media.

To test this hypothesis we inoculated a streptococcus (*fæcalis*) and the Bundaberg staphylococcus together into a rubber-capped bottle of toxin-antitoxin mixture of Batch 86 and incubated it at 80° F. One cubic centimetre of the mixture was injected on the first, second, third, sixth, and ninth days into guinea pigs and in no case were any symptoms produced other than slight temporary loss of weight and local ulceration. On the tenth day both organisms were recovered by plating.

B. proteus X 19 was inoculated into a rubber-capped bottle of Batch 78A and after 24 hours the Bundaberg staphylococcus was also inoculated in a dose of about 1,000 organisms. Injections into guinea pigs in doses of 0·5 c.cm. were made on first, second, third, fourth, fifth and eighth days. Beyond some local ulceration and loss of weight no symptoms were noted, though the animal inoculated on the fifth day was very slow in recovering its original weight. After nine days' incubation at room temperature (about 23° C.) both organisms were recovered by plating. Another bottle was inoculated with *B. proteus X 19* alone in a dose of about 1,000 organisms. A similar series of animal injections were made without positive results. These experiments are of particular interest in that an organism with strong proteolytic powers apparently failed to cause any obvious dissociation of diphtheria toxin from toxin-antitoxin mixture.

Other streptococci tested for powers of growth and survival for ten days in toxin-antitoxin mixture were a hæmolytic strain from a puerperal case and one from a case of scarlet fever. Guinea pigs were inoculated at the intervals described above and none showed any symptoms. The organisms were recovered from the bottles at the end of the experiment. Similar experiments were later carried out with *B. tetani* (Appendix 20). In view of these findings it did not seem worth while pursuing this line of inquiry further.

No experiments were made with yeasts as we had been unable to recover them by culture either on the many media in which the staphylococcus grew, in bottles of toxin-antitoxin, or later, on special media.

It is very unlikely that any pathogenic organisms of microscopic size other than the staphylococci were at any time present in any considerable numbers in Dr. Thomson's bottle. If, as is possible, a few other contaminating organisms got in, they should, if they had grown, have been recoverable by culture when the bottle reached us.

We cannot exclude the presence of a pathogenic filter-passing organism in the Bundaberg bottle, but it is exceedingly improbable that any such organism would have grown in so feebly nutrient a medium. The only organism other than the staphylococcus of whose presence in Dr. Thomson's bottle we have evidence, is the yeast which we were unable to recover and which did not appear to have multiplied to any extent in the bottle. Unlike the case of the staphylococcus, we cannot exclude the possibility that this organism was introduced by Dr. McKeon in taking a sample (Appendix 18).

APPENDIX 22.

Report of Serological and Other Characteristics of Staphylococci Associated with the Bundaberg Investigation.

Dr. F. M. Burnet, of the Walter and Eliza Hall Institute, who worked out for us the relationship of the various staphylococci isolated in these experiments, contributes the following summary of his results:

Material.

Numerous cultures from the original bottle used by Dr. Thomson showed the same organism, and for stock a single colony was taken from one of the original serum agar slopes made on February 3, 1928. This is named "Bundaberg" in the following.

From anaerobic glucose broth cultures inoculated from the original bottle a number of *aureus* colonies was obtained and stock culture $B.T_2$ was developed from one of these. Reasons will be given for considering this a variant of the original Bundaberg strain.

Other material was as follows: (1) Agar slope cultures of organisms from abscesses in children's arms—

B.D., W.S., V.T.C. .. .. } From Dr. Richards, Rockhampton.
B.P., E.D. (A) .. .. } From Dr. Tebbutt.

These were all of the same appearance, white with a faint creamy tinge in young cultures, as the Bundaberg staphylococcus. (2) Agar slope culture from abscess of buttock (E.D. (B)). (3) Staphylococci isolated from Dr. Thomson's water bottle (W.B.) and rainwater tank (Tank) by Dr. Tebbutt on agar slopes. (4) Several strains of staphylococci obtained from bottles of toxin-antitoxin mixture injected with air in Bundaberg, *viz.*, F1 (*albus*), F3 (*albus*), F8 (*albus*), F7 (*aureus*). (5) Two strains isolated from bottles into which Dr. Thomson had injected air in removing sample for demonstration of his technique at Bundaberg, *viz.*, T2.A. (*albus*), T2.B. (*albus*).

Several sera eventually pooled were made by immunizing rabbits against "Bundaberg," the final titre being approximately 1,280.

Sera were also made against strain $B.T_2$.

Agglutinating emulsions were made from all the strains available, in the usual way—growth on agar and emulsification in formalized saline to an opacity corresponding to 2,000 millions per c.cm.

These were tested with each serum with the results tabulated below. In the same table are shown the colour and the hæmolytic power of the various strains on human blood agar.

Strain.		Serum "Bundaberg."	Serum $B.T_2$.	Colour.	Hæmolysis on Human Blood Agar.
Bundaberg		1,280	320	—	−
	$B.T_2$. ..	40	640	*aureus*	+
Cultures from arms of survivors	B.D. ..	640	40	—	−
	E.D. (A)..	640	80	—	−
	B.P. ..	640	80	—	−
	V.T.C. ..	640	80	—	−
	W.S. ..	640	40	—	−
Tank		0	—	*albus*	−
W.B.		0	0	*albus*	
Cultures from air in T.A.T. bottles	F1 (*albus*)	40	40	*albus*	+
	F3 (*albus*)	40	0	*albus*	+
	F8 (*albus*)	40	0	*albus*	+
	T2.A ..	40	0	*albus*	+
	T2.B ..	40	640	*albus*	−
	F7 (*aureus*)	40	40	*aureus*	−
	E.D. (B)..	640	160	*aureus*	+

It will be seen that the cultures from the arms of the various survivors form a compact group differing in no important point amongst themselves. They are identical with the Bundaberg staphylococcus except in the one respect that they agglutinate to a consistently lower titre with the heterologous serum $B.T_2$.

Absorption experiments with the two sera and their homologous strains indicate that $B.T_2$ possesses two antigens, one in common with "Bundaberg," the other specific. This is compatible with the view that $B.T_2$ is a variant of "Bundaberg" analogous to a partly "rough" strain, although in the present absence of knowledge concerning the serological changes with staphylococcal variation no stress can be laid on this interpretation.

The serum "Bundaberg" agglutinated several typical pyogenic *aureus* strains from other sources to full titre and this organism must be regarded as a serologically typical pyogenic strain as first described by Kolle and Otto, and confirmed (on the whole) by Julianelle, Hine and others. The various strains derived from air and water at Bundaberg seem to belong to the unrelated non-pyogenic strains with the possible exception of T2B, which is closely related to the presumed variant of "Bundaberg" $B.T_2$.

The difference in the history of the strains is probably sufficient to account for the slight distinction in serological behaviour between "Bundaberg" and the strains derived from the children's arms.

The identity of all these strains is also supported by two incidental lines of evidence. They all show under appropriate conditions Müller's phenomenon on human blood agar, *i.e.* an appearance of punctate hæmolysis in a zone extending several millimetres beyond the limits of growth of the organism. This phenomenon is rather rare amongst staphylococci, two others only of about a dozen tested showing it.

In the second place a bacteriophage was obtained weakly active against strain "Bundaberg." This was tested against a number of staphylococcal strains with the results tabulated, the number of +'s indicating the relative degree of lysis on agar.

"Bundaberg"	.. ..	+++	E.D. (Buttock) .. .. –
$B.T_2$.	–	F_7. aureus	–
B.D.	++	Six staphylococcal strains not connected with the Bundaberg investigation. All	–
B.P.	++		
W.S.	++		
V.T.C.	+++		
E.D. (A)	+++		

In all cases where lysis occurred it was incomplete and was followed by secondary growth. Staphylococcal bacteriophages fall into two types, one type is active against almost all varieties of staphylococci with the exception of specifically resistant variants, the other is practically confined to the homologous strain (d'Herelle, Gratia, Callow and others). The sensitivity of all the arm cultures and these only to the phage active against "Bundaberg" is, therefore, very strong evidence of their identity with this strain.

In regard to the strain E.D. (buttock), its characteristics (*aureus*, hæmolytic, not sensitive to phage) are sufficient to separate it from the "Bundaberg" group despite its practical serological identity. It resembles in all respects a normal pyogenic *aureus* and is serologically distinct from the *aureus* variant obtained from "Bundaberg."

Later work has shown that in its agglutination and absorption reactions, its sensitivity to bacteriophage and its reactions on blood agar the Bundaberg strain is identical with Hine's Type II (*Lancet*, 1922), strain 1,393 of the national collection of type cultures.

Conclusions.

The organism "Bundaberg" obtained from the implicated bottle is identical with the staphylococci recovered from the childrens' arms in the cases of B.D., W.S., B.P., E.D. and V.T.C.

None of the organisms obtained by injection of air into toxin-antitoxin bottles (or from the water supply) at Bundaberg are of this type.

The staphylococcus obtained from the lesion in the buttock (E.D. (B)) is also not of the type.

APPENDIX 23.

The Pathogenicity of the Bundaberg Staphylococcus to Laboratory Animals.

The experiments detailed in Appendix 18 do not suggest that this organism is, with the single exception of the rabbit, highly pathogenic for the ordinary laboratory animals. A large number of experiments with guinea pigs and a few with mice, rabbits and monkeys have been concerned with the pathogenicity of the staphylococcus when grown in toxin-antitoxin mixture (Appendix 26).

In this place we have gathered together a few of the results of inoculation of cultures and emulsions from various media which indicate the somewhat variable pathogenicity of the organism for rabbits and its low pathogenicity for monkeys, guinea pigs and mice.

Subcutaneous and intramuscular injections in these animals do not produce fatal results. These can only be obtained by intravenous or intraperitoneal inoculation.

In mice of about twenty grammes weight inoculated intraperitoneally with a saline emulsion from a twenty-four hours agar slope culture, 5,000 million organisms kill in from six to eight hours, 500 million in twenty hours, while with doses of 50 millions the animals survive.

In guinea pigs of 250 to 300 grammes weight, 500 to 1,000 million organisms cause a small local abscess when injected subcutaneously. There is also a temporary decline in body weight. Doses of 200 million organisms injected intravenously kill on about the fourth day. A fatal result is produced in six days by injecting intravenously 0·5 c.cm. of a twenty-hour broth culture while the same culture in twice the dose injected intramuscularly fails to kill and causes only local abscesses.

In rabbits weighing approximately one kilogram, 200 and 400 million organisms when injected intravenously killed on the second and sixth day, and ten million organisms killed one of two animals on the tenth day. Five hundred and 1,000 million staphylococci injected subcutaneously caused only a small local lesion and the same doses given intramuscularly gave only local effects with small loss of weight. Comparing intravenous and intramuscular injections with a twenty-hour broth culture, doses of one cubic centimetre by the latter route caused only local lesions and loss of weight, while half this dose given intravenously killed on the third day. In these late deaths the usual pyæmic lesions in the kidneys were found at autopsy.

Intravenous injections of broth cultures of the staphylococcus were also made into a number of young monkeys.

Monkeys 5, 6 and 7 had earlier received subcutaneous injections of cultures of the staphylococcus in toxin-antitoxin mixture. The results of these injections are described in Appendix 26.

Monkey 5, which weighed 2·1 kilograms, received one cubic centimetre of a twenty-four hours culture which had been inoculated with one platinum loopful of a standard emulsion from an agar slope, which was itself a subculture from a culture of the staphylococcus grown in a mixture of monkey blood and toxin-antitoxin. No effect was apparent in thirteen days and the weight was then 2·5 kilograms.

Monkey 6, which weighed 2·27 kilograms, received two cubic centimetres of a twenty-four hour broth culture inoculated direct from defibrinated monkey blood, in which the staphylococcus had grown for three days, after passage through decreasing dilutions of monkey blood with toxin-antitoxin mixture. A slight rise in temperature was observed next day, but no obvious illness resulted.

After nine days the weight was found to be 2·13 kilograms. The lymph glands in the groin were enlarged though the local lesions resulting from the previous subcutaneous inoculations had quite disappeared.

Monkey 7, which weighed 2·35 kilograms, received two cubic centimetres of the same broth culture as monkeys 6 and 10, but after forty-eight hours' incubation. A slight rise of temperature also occurred next day, but no obvious illness resulted for eight days when his weight was found to be 2·38 kilograms and he appeared well. Two days later he went off his food. On the eleventh day he was obviously ill, eating very little, temperature only 36° C.. He was found dead, but still warm the next morning. He had not had diarrhœa. Weight 1·9 kilograms. Autopsy revealed a remarkable picture of acute generalized staphylococcal infection. There were hæmorrhages into the skin and serous membrane and small vegetations on the mitral valve. The spleen was enlarged and firm and showed recent firm infarcts of large size. The liver was congested but showed no focal lesions. The kidneys showed hæmorrhages and one recent infarct and were much swollen. The retroperitoneal and mesenteric lymph glands were markedly swollen and of dark colour. Cultures gave staphylococci resembling "Bundaberg" from heart blood, liver, spleen, lymph glands (several), and kidney all in pure culture.

The histological examination of the liver shows congestion of the sinusoids, central veins and portal branches, accumulations of small and large mononuclear cells in some of the portal tracts, and occasional small areas of focal necrosis in or near portal tracts.

In the spleen there is much engorgement of the pulp and relatively small Malpighian bodies, many of which show central necrosis, sometimes involving thrombosis of the arteriole.

All the glands examined show hæmolymph characters. They are extremely engorged and œdematous with dilatation especially of the medullary sinuses which contain abundant large phagocytic endothelial cells stuffed with red corpuscles. A large number of the lymph follicles have necrotic centres which, like those seen in the liver and spleen, are mostly in process of absorption.

In many points there is a close resemblance in the condition of these glands to some of those examined from the children at Bundaberg, and the appearances observed suggest the possibility that some of these also may be of hæmolymph type, though the difficulty or impossibility of recognizing red cells in sections of engorged vessels in these cases allows no security of opinion.

Gram's stain reveals abundant staphylococci distributed everywhere through the tissues examined. They are seen in the blood stream, occasionally plugging small vessels in the glands and spleen, in phagocytes, in the swollen Küpffer cells and even in the liver cells themselves. It is notable that their presence does not appear at any point to excite any focal reaction and nothing in the nature of abscess formation is seen. Polymorphs are not a conspicuous feature of any of the sections.

Monkey 10, weighing 2·75 kilograms, received 1·0 c.cm. of the same broth culture (twenty-four hours) as Monkey 5. The temperature response following the injection may be regarded as typical in this series of experiments. Before inoculation the rectal temperatures of all the animals lay between 38·5° C. to 38·75° C. On the second and third days the temperatures in Monkey 10 were 38·75° C. and 39° C. and the animal was sulky. On the sixth and the following three days the temperatures were 39·2° C., 38·9° C., 38·9° C. and 38° C., and the animal looked well and was active and eating well. Its weight on the fourteenth day was 2·6 kilograms. There were then enlarged glands in the groin particularly on the right side, but no other signs or symptoms of illness.

Monkey 11, weighing 1·6 kilograms, temperature 38·3° C., received 2·0 c.cm. of the same broth culture (forty-eight hours) as Monkey 7. This animal was already underweight and sullen. On the second day the temperature was 38·1° C., on the third 38·6° C., and on the fourth 37·8° C. It developed diarrhœa and on the seventh day the temperature was still lower. It refused food and was moribund on the ninth day. No abscesses were discovered at autopsy and no lesions of the internal organs other than enlargement of the mesenteric lymph glands. Cultures from the heart blood and pericardial fluid were sterile. The cultures from the spleen when plated grew a few colonies resembling the Bundaberg staphylococcus. Death was apparently due to entero-colitis.

Monkeys 7 and 11 were the only animals in this series which succumbed, and of these Monkey 7 alone showed a generalized infection. Monkey 11 received the same intravenous injection, but not the preliminary subcutaneous ones and died within the period of development of the disease in Monkey 7, possibly from an intercurrent bowel affection. There is a possibility that the previous subcutaneous injection of a four-day culture of the staphylococcus in toxin-antitoxin mixture may have determined the fatal issue, but it seems more probable that the important factors were the larger numbers of organisms later introduced (forty-eight hours culture) and the individual susceptibility of the animal in question.

All the experiments recorded above were carried out two to three months after the organism had been isolated, without passage through any animals in the meanwhile. Some earlier experiments with rabbits recorded in the table below give indications of much greater pathogenicity.

For the ordinary laboratory animals this staphylococcus is of about average virulence. Its virulence for rabbits is somewhat variable. The first subculture from the Bundaberg bottle showed surprising virulence and killed a large rabbit so quickly as to give no time for the production of any obvious lesions.

Two of these animals which died rapidly, convulsed continuously before death. This symptom was never seen in the animals which died after longer periods with a less acute illness.

EFFECT OF INTRAVENOUS INJECTION OF THE BUNDABERG ORGANISM INTO RABBITS.

	Body Weight in Kilograms.	Dose.	Result.
1	1·9	0·1 c.cm. glucose brain broth culture 11 hours (inoculated from 78A B* 24 hours after inoculation from B.T.A.T.)	Died with convulsions in 11 hours; *p.m.*, hæmorrhages in lumbar muscles, meningeal congestion, staphylococci in pure culture from heart blood.
2	1·5	1·0 c.cm. 24-hours glucose brain broth heart blood culture from 1	Dead in 22 hours; necrotic lesions in liver, pericarditis, infarction in both kidneys, hæmorrhages in ribs at costochondral junction.
3	1·4	0·5 c.cm. of same ..	Dead in 23 hours; infarcts in kidneys; necrotic lesions in liver.
4	1·45	0·2 c.cm. 6-hours glucose brain broth culture of heart blood of 2	Survived.
5	1·3	0·5 c.cm. of same ..	Survived.
6	1·4	0·2 c.cm. 27-hours brain broth culture of heart blood of 2	Death in 13 hours with convulsions; punctate necrosis in liver, infarct in right kidney, staphylococcus isolated in pure culture from heart blood.
7	1·5	0·25 c.cm. glucose brain broth (sub of original culture)	Died in 5 days; septic infarcts in kidneys and necrotic areas in liver.
8	1·2	0·25 c.cm. serum from heart blood taken by intracardiac puncture from 7 (on third day) and incubated 24 hours	Died in 24 hours; no lesions.
9	1·3	0·25 c.cm. 48-hours culture of heart blood of 7 in glucose brain broth	Survived.

* 78A B = Bottle of Batch 78A inoculated from the original Bundaberg bottle (B.T.A.T.) and incubated at room temperature (Appendix 18).

APPENDIX 24.

Production of a Skin-reacting Toxin from the Bundaberg Staphylococcus.

It is known that filtrates from staphylococcal cultures may produce positive skin reactions when injected intradermally in susceptible human individuals. This offered a suggestion for an attempt to demonstrate the formation of an active toxin by the Bundaberg staphylococcus, and Dr. F. M. Burnet has kindly carried out experiments for us of which a brief *résumé* is given here.

If a staphylococcal toxin played any important part in causing the Bundaberg deaths, it must have been produced within a few hours of the introduction of the organisms into the body. For this reason the tests were all made with filtrates from young cultures seven to eight hours old. Preliminary experiments were done with cultures in a mixture of toxin-antitoxin and human blood. In susceptible individuals a positive skin test was obtained with 1 in 500 or 1 in 1,000 dilutions of a filtrate from such a culture. The reaction showed as an area of erythema about 2 cm. in diameter with occasional slight subjacent swelling in all respects similar to a positive Schick or Dick test. Later work showed that similar reactions were obtainable when the cultures were grown in human blood broth and this medium was used for most of the experiments.

Five cubic centimetres of freshly drawn human blood were added to ten cubic centimetres of citrated broth. The tube was heavily inoculated from a young agar culture of the required staphylococcal strain and incubated at 37° for seven or eight hours. The supernatant fluid was then pipetted off from the deposited corpuscles and after dilution with saline (1 in 3) was passed through a Seitz filter. The filtrate was tested for sterility and diluted to 1 in 500 as required. In addition to the Bundaberg strain another staphylococcus (S.I.) was used. This was a typical pyogenic aureus strain derived from a human skin lesion.

A mixed group of individuals was tested with these filtrates by the injection of 0·2 c.cm. of 1 in 500 dilutions with the following results:

	S.I.	Bundaberg.
Number tested	36	44
Number positive	16	20
Number negative	17	18
Number doubtful	3	6

In each instance about half the individuals tested were sensitive to the filtrate at this dilution.

The skin-reacting substances present in filtrates from the two organisms seem to be distinct one from the other. The degree of reaction noted in each of the individuals who received both filtrates is shown in the following table:

Subject.	B.	S.I.	Subject.	B.	S.I.
C. K. ..	−*	++	D. A. ..	++	++
F. B. ..	++	++	G. B. ..	+	++
B. S. ..	++	++	T. C. ..	−	−
H. W. ..	+	++	T. D. ..	+	+
E. K. ..	−	++	A. H. ..	++	−
N. A. ..	−	±	S. L. ..	−	−
K. A. ..	−	−	C. O. ..	++	−
K. B. ..	+	−	F. P. ..	++	+
J. C. ..	++	−	G. S. ..	++	−
B. D. ..	−	−	J. B. ..	±	++
R. G. ..	+	−	L. C. ..	−	++
A. J. ..	−	−	J. H. ..	−	−
G. L. ..	±	−	M. J. ..	+	++
V. M. ..	±	±	M. R. ..	+	+
G. M. ..	−	−	N. S. ..	−	−
R. S. ..	±	−	E. H. ..	+	−
F. T. ..	−	±	E. M. ..	++	+
L. A. ..	−	+	R. W. ..	+	++

++ denotes a reaction 1 inch or more in diameter.
+ denotes a reaction ½ inch to 1 inch in diameter.
± denotes a reaction less than ½ inch in diameter.

It will be seen that occasionally either filtrate may give a strong reaction while the other is completely inert.

Filtrates from staphylococcal cultures in plain broth grown for the same length of time are definitely less active than those from blood broth cultures although some skin-reacting substance is produced. It seemed possible that differences in the amount of active agent produced might be found according to whether the blood used in the medium were derived from susceptible or non-susceptible individuals. The "Bundaberg" staphylococcus was, therefore, grown in citrated broth (*a*) with blood from a positively reacting individual, (*b*) from a non-reacting subject, (*c*) without addition of blood.

Filtrates were tested on a susceptible subject with the following results:

	Dilution.	Diameter of Reaction.
Filtrate made with "susceptible" blood ..	1 in 1,000	2·5 cm.
	1 in 2,000	Nil
Filtrate made with "non-susceptible" blood	1 in 1,000	2·2 cm.
	1 in 2,000	2·2 cm.
Filtrate from culture without blood	1 in 1,000	Nil
	1 in 2,000	Nil

If anything, the "non-susceptible" blood produces more of the skin-reacting substance, but it is doubtful if the difference is significant.

We have further attempted in two of the workers in this Institute, F.B. and B.S., to ascertain whether the serum from N.W. (Case 8) who had no symptoms following the injection on January 27 could protect in sensitive individuals in a dilution of 1 in 20 against 1 in 1,000 and 1 in 400 of the toxin (filtrate of Bundaberg staphylococcus blood broth cultures). The serum itself in this dilution produced a very slight reaction which is not surprising in view of its age and the possibility of contamination. The dilution was, of course, filtered before use. As controls in this experiment we injected 1 in 1,000 of the toxin alone and 1 in 1,000 and 1 in 400 of the toxin mixed with a 1 in 10 dilution of the sera of R.G. and J.C. (both known skin reactors).

	F.B.	B.S.
1/1000 B. Filtrate	++ (2·0 cm.)	++ (2·2 cm.)
1/1000 B. Filtrate 1/20 N.W.	+ (1·5 cm.)	+ (vague)
1/400 B. Filtrate 1/20 N.W.	+++ (3·1 cm.)	++ (2·5 cm.)
1/1000 B. Filtrate 1/10 J.C.	++ (2·2 cm.)	++
1/1000 B. Filtrate 1/10 R.G.	+ (1·5 cm.)	± (2·2 cm.)

These preliminary observations are too few for any stress to be laid upon their interpretation. The Bundaberg staphylococcus grown under appropriate circumstances can produce a substance whose activity is shown in specific skin reactions in a proportion of human individuals. It may be that these reactions indicate differences in susceptibility to the general pathogenic activity of the organism. This interpretation would be analogous to the current view of the relationship between the Dick test and the variation of pathogenicity of the streptococcus of scarlet fever human individuals. A further point of resemblance is afforded by the absence of any toxic effects when skin-reacting filtrates are injected in doses of 5 c.cm. (undiluted) into laboratory animals (monkey, guinea pig and rabbit).

If the Bundaberg staphylococcus were responsible for the illness and deaths which followed its subcutaneous injection on January 27, these observations may be held to suggest a partial explanation of the failure of some of the children to show any symptoms. It is obvious that the crucial experiment of ascertaining whether the survivors at Bundaberg are or are not sensitive to the skin-reacting substance cannot be carried out.

APPENDIX 25.

The Effect of the Bundaberg Staphylococcus on Toxin and Antitoxin Separately.

For this purpose it was decided to use toxin diluted to about the same concentration as in Batch 86, to which normal sterile horse serum was added so as to make the protein content of the mixture about equal to that of the toxin-antitoxin mixture. Similarly antitoxin was made up containing approximately one unit per c.cm. with sugar-free broth to take the place of toxin.

The toxin used was pooled 21A and 21D of the Commonwealth Serum Laboratories. The antitoxic serum used was also prepared by the Commonwealth Serum Laboratories and consisted of mixed batches of returned sera similar to those used in the preparation of Batch 86.

It was first of all necessary to determine the approximate L+ dose of the toxin and the titre of the antitoxin.

For the determination of the L+ dose a number of guinea pigs weighing 250 grammes was inoculated subcutaneously with mixtures consisting of varying amounts of toxin with 1·0 unit of standard antitoxin. The mixtures were made up to a volume of 4·0 c.cm. with saline and allowed to stand one hour before injection.

The results of these tests are shown in Table I.

TABLE I.
DETERMINATION OF L+ DOSE OF TOXIN.

Number of Animals.	Dose of Toxin.	Result.
	c.cm.	
One..	0·200	Infiltration—loss of weight until seventh day. Survived.
One..	0·225	Infiltration—loss of weight until seventh day. Survived.
Five	0·250	Died 64 hours, 87 hours, 94 hours, 94 hours and 101 hours.
Two	0·260	Died 66 and 67 hours.
Two	0·275	Died 77 and 72 hours.
One..	0·300	Died 44 to 64 hours.
One..	0·325	Died 44 to 64 hours.
One..	0·350	Died 42 hours.

The L+ dose was, therefore, about 0·250 c.cm. for this toxin.

This dose of toxin was now put up with varying doses of 1 in 1,000 dilution of the antitoxic serum to be used. The mixtures were made up to 4·0 c.cm. with saline and allowed to stand one hour before injection into guinea pigs of 250 grammes weight. The results are shown in Table II.

TABLE II.
TITRATION OF ANTITOXIN.

Number of Animals.	Dose of 1 in 1000 Serum.	Result.
	c.cm.	
Two	0·71	Died 54 to 63 hours.
Two	0·74	Died 58 to 72 hours.
Two	0·77	Died 58 to 65 hours.
Two	0·80	Died 60 to 87 hours.
One..	0·85	Died seventh day.

We therefore regarded this antitoxin as containing approximately 1,250 units per c.cm.

The mixtures were, however, made up before the results of this last titration were complete and were as follows:

A. Toxin Mixture—

Toxin	20 c.cm.
Horse serum 1 in 20 in saline	1·5 c.cm.
Saline	80 c.cm.

B. Antitoxin Mixture—

Sugar-free broth	10 c.cm.
Antitoxin 1 in 20 in saline	1·5 c.cm.
Saline	90 c.cm.

In spite of the too small proportion of antitoxin in B, when 1·5 c.cm. was added to an equal quantity of A. and the mixture injected subcutaneously into a guinea pig, the animal showed no infiltration and gained weight steadily.

The mixtures were made up with full aseptic precautions and proved to be sterile in bulk. Their pH was approximately 7·0.

They were now bottled in sterile amber glass bottles (obtained from used samples of Batch 86) containing approximately 10 c.cm. each and covered with rubber caps from the same source. They were tested for sterility separately by inoculation of 0·5 c.cm. into 10 c.cm. of broth, and all but one or two bottles were found to be uncontaminated.

They were now inoculated with the Bundaberg staphylococcus and incubated at 80° F. The dose inoculated was estimated by plating to be 7,000 cocci. Growth took place in all the bottles (see Appendix 26).

Each day in the earlier days of the experiment and later at longer intervals the titre of toxin and of antitoxin was determined, and at the beginning and end of the experiment the original bulk was similarly tested. In the case of antitoxin a further control was provided by incubating an uninoculated rubber-capped bottle seven days at 80° F. Antitoxin was titrated by the intradermic method using 0·1 c.cm. of the following range of dilutions of the mixture—1 in 12·5, 1 in 16·6, 1 in 25, 1 in 33·3 and 1 in 50, corresponding to 0·008, 0·006, 0·004, 0·003 and 0·002 unit of antitoxin if the mixture had contained 1·0 unit of antitoxin per c.cm. An equal volume of standard toxin solution was added to each dilution in a dose of 0·0009 c.cm. in each 0·1 c.cm. Protection experiments were also carried out using some or all of the following dilutions of B—1 in 80, 1 in 70, 1 in 60, 1 in 50, 1 in 40, 1 in 30, 1 in 25 and 1 in 20. One c.cm. of each of these was mixed with the C.L.D.[1] of toxin, 0·01 c.cm. in 1 c.cm. of saline and the mixture after standing an hour was injected subcutaneously into guinea pigs weighing between 240 and 260 grammes. Animals which survived were discarded on the twentieth day or later.

Toxin was titrated using the toxin mixture A. in dilutions of 1 in 24, 1 in 22, 1 in 20 and 1 in 18 for intradermic injections of 0·1 c.cm. mixed with 0·1 c.cm. of saline containing 0·002 unit of standard antitoxin. The total volume of the injections in the intradermic tests was in every case 0·2 c.cm.

The content of toxin in A. was also checked by injecting 1 c.cm. of 1 in 20 of the mixture equivalent to 0·01 c.cm. of toxin if no deterioration had occurred, and occasionally in a dilution of 1 in 16·6 and 1 in 14·3 to indicate the extent of any loss of activity.

The results of these experiments are set out in Tables III and IV. Judged by the intradermal test no appreciable destruction of toxin occurred during eighteen days' incubation. The guinea pigs injected with what should have been equivalent to the C.L.D. of toxin did not all die, so that it is possible that a small loss of activity resulted from the growth of the staphylococcus. On the eighteenth day 1 c.cm. of 1 in 20 of the bottle containing the staphylococcus killed both animals into which it was injected on the third day.

Mr. Holden had been able to recover a trace of iodine (Appendix 19) in the washings from the interior of the Bundaberg bottle. This amounted to 0·003 mgm. in 0·075 c.cm. of mixture left in the bottle (estimated by weighing). This amount corresponded to 0·04 mgm. per c.cm. of mixture and had evidently been introduced into the bottle by successive punctures through the rubber cap. We attempted to ascertain whether the presence of iodine had any effect. The staphylococcus inoculated into a bottle of

[1] C.L.D. is used to signify "certain lethal dose."

TABLE III.
TITRATION OF TOXIN ON SUCCESSIVE DAYS AFTER INOCULATION.

	$L^+/_{500}$ Dose Intradermic.	Inoculation of Mixture into Guinea Pigs Subcutaneously.		
		Number of Animals.	Dose.	Results.
Original mixture	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	Died on the third and fifth days.
After 49½ hours incubation	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	One died on the sixth day, the other survived gaining weight by eleventh day.
After 75 hours	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 16·6	Both died on the third day.
After 99 hours	0·1 c.cm of 1 in 22	2	1 c.cm. of 1 in 16·6	One died on the fifth day, the other regained body weight by the fifteenth day.
After 123 hours	0·1 c.cm. of 1 in 22			
After 7 days	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	One died on the fifth day and one regained body weight by twelfth day.
After 12 days	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	Died on the third and twenty-sixth days.
		2	1 c.cm. of 1 in 16·6	Died on the third and fifth days.
After 18 days	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	Died on the fourth and seventh days.
	(0·1c.cm.of1in24still	2	1 c.cm. of 1 in 16·6	Died on the third and sixth days.
	nearly necrosis level)	2	1 c.cm. of 1 in 14·3	Died on the third and fourth days.
Original mixture at end of experiment	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	Both dead on third day.
	(0·1 c.cm. of 1 in 24	2	1 c.cm. of 1 in 16·6	Died on third and fourth days.
	nearly necrosis level)	2	1 c.cm. of 1 in 14·3	Both dead on third day.
Original mixture + Iodine, incubated 8 days	0·1 c.cm. of 1 in 20	2	1 c.cm. of 1 in 20 ..	One died on fourth day and one regained weight by the thirteenth day.
		2	1 c.cm. of 1 in 16·6	Both died on the fourth day.
		2	1 c.cm. of 1 in 14·3	Both died on the fourth day.
Original mixture + Iodine, incubated 12 days	0·1 c.cm. of 1 in 22	2	1 c.cm. of 1 in 20 ..	One died on eighth day, the other regained body weight by thirteenth day.

TABLE IV.

TITRATION OF ANTITOXIN ON SUCCESSIVE DAYS AFTER INOCULATION.

	Intradermal Titration. $^1/_{500}$ Unit A.T. in 0·1 c.cm. of—	Injection of 1 c.cm. of varying Dilutions of Antitoxin Mixture with 1 c.cm. containing 0·01 Toxin.		
		Number of Animals.	Dilution of A.T. Mixture.	Results.
Original mixture before inoculation..	1 in 50	3	1 in 50	Extensive infiltration and later ulceration; original weight regained by fifteenth, seventeenth and twenty-third days.
			1 in 60	One died on the seventh, one on the twenty-sixth day, and one had regained its original weight by the twenty-first day.
		3	1 in 70	Died on fourth, sixth and eighth days.
		3	1 in 80	One died on the ninth and two on seventh day.
After 49½ hours	1 in 50	2	1 in 25	No infiltration; steady gain in weight.
		2	1 in 50	Extensive infiltration and necrosis; weight regained by twenty-first day.
		2	1 in 60	Both died on seventh day.
		2	1 in 70	Both died on ninth day.
		2	1 in 80	Died on seventh and ninth days.
After 75 hours	1 in 50 (1 in 33·3 gives more marked reaction than earlier)	2	1 in 20	No infiltration; steady gain in weight.
		2	1 in 30	Slight infiltration; no loss of weight.
		2	1 in 40	Infiltration; loss of weight—regained by nineteenth day.
		2	1 in 50	Infiltration; loss of weight—not regained by twenty-first day.
		2	1 in 60	Died on fourth and seventh days.
After 99 hours	1 in 50 (1 in 33·3 reaction nearly necrosis level)	2	1 in 40	Infiltration, necrosis; regained weight by nineteenth day.
		2	1 in 50	Infiltration, necrosis; regained weight by seventeenth day.
		2	1 in 60	One died on the seventh and one regained body weight by twentieth day.
		2	1 in 70	Died on sixth and eighth days.
After 123 hours	1 in 50 (1 in 33·3 nearly necrosis level; reaction with 1 in 25 persists 4 days)	2	1 in 40	Extensive infiltration and necrosis; one regained weight by eighteenth day.
		2	1 in 50	One died on the seventh and the other had not regained weight by eighteenth day; paralysis of left hind limb.
		2	1 in 60	One died on sixth and the other regained weight by fourteenth day.
After 7 days	1 in 50 (reactions as after 123 hours)	2	1 in 25	Slight infiltration; slight loss of weight in one only.
		2	1 in 40	Infiltration, necrosis; body weight regained by sixteenth day.
		2	1 in 50	One died on the eighth and the other regained body weight by sixteenth day.
		2	1 in 60	Both died on fifth day.
After 12 days	Bewteen 1 in 50 and 1 in 33·3	2	1 in 20	Definite slight infiltration, but no loss of weight.
		2	1 in 30	Infiltration; no loss of weight.
		2	1 in 40	Infiltration, necrosis; body weight regained by ninth day.
		2	1 in 50	One died fourth day and one had infiltration and necrosis and regained weight on seventh day.
		2	1 in 60	Both died on twenty-fourth day.
After 18 days	1 in 33·3	2	1 in 20	No infiltration; steady gain in weight.
		2	1 in 30	Infiltration; steady gain in weight.
		2	1 in 40	Infiltration and necrosis; body weight regained by fifth and twelfth days.
		2	1 in 50	Died on the seventh and eighth days.
		2	1 in 60	Died on the fourth and eighth days.
Control bottle uninoculated 7 days incubated	1 in 50 (1 in 33·3 gives good reaction nearly to necrosis level)	2	1 in 20	Very slight infiltration; slight loss of weight in one only.
		2	1 in 30	Infiltration; steady gain in body weight.
		2	1 in 40	Infiltration; body weight regained by seventh day.
		2	1 in 50	Infiltration, necrosis; body weight regained in one by eighth day, but in the other was not regained by the eighteenth day.
		2	1 in 60	Died fourth day and seventh day.
Control stock at end of experiment ..	1 in 50	2	1 in 30	Infiltration; one regained original weight on fifth and the other on twelfth day.
		2	1 in 40	Infiltration and necrosis; both regained weight by twelfth day.
		2	1 in 50	One died on the twenty-sixth day, the other regained weight by twelfth day.
		2	1 in 60	One died on the seventh day and the other had not regained weight by the twelfth day.
		2	1 in 70	One only regained weight by the twelfth day.

A. containing iodine in half the concentration observed by Mr. Holden grew satisfactorily and the intradermal tests indicated a slight but definite decrease in the activity of the toxin.

The effects of the growth of the Bundaberg staphylococcus on antitoxin were somewhat greater than those observed in the case of toxin. The intradermal tests indicated a decrease of about one-third in antitoxic value after eighteen days.

The protection experiments confirmed this finding though they suggest that the decrease is about $^1/_5$ and that some falling off of antitoxic activity takes place in this dilution at 5° C. even in the absence of bacterial contamination. One cubic centimetre of a dilution of 1 in 50 of the original mixture protected against the C.L.D. of toxin. After eighteen days at 5° C. 1 c.cm. of 1 in 40 was required. The bottle incubated seven days was unfortunately contaminated, though it was still clear and grew only a few colonies on plating. This control was, therefore, abandoned. Of the material in which the Bundaberg organism was growing, 1 c.cm. of 1 in 40 was necessary after eighteen days' incubation for protection against the C.L.D. of toxin.

It cannot be inferred from these experiments that the Bundaberg organism is capable of destroying antitoxin preferentially in toxin-antitoxin mixtures. In our experimets (*vide* Appendix 26) we have been unable to obtain any evidence that such is the case.

APPENDIX 26.

On the Cultivation of Staphylococcus in Toxin-Antitoxin, on the Possibility of Dissociation of Toxin by this Method, and on the Pathogenicity of Staphylococci Grown in this Medium.

Toxin-antitoxin as prepared by the Commonwealth Serum Laboratories cannot compare with plain broth as a

culture medium for staphylococci. The particular batch (No. 86) with which we are mainly concerned was made up to contain in 1 c.cm. 80% of an L+ dose of toxin and one unit of antitoxin. The L+ dose of the toxin used was 0·325 c.cm. and the titre of the antitoxin 1,350 units per c.cm. Therefore, each c.cm. of toxin-antitoxin should contain (i) 0·260 c.cm. of toxin (*i.e.* broth in which first colon bacilli, to render sugar free, and then diphtheria bacilli have been grown), (ii) 0·00073 c.cm. of antitoxin (*i.e.* horse serum globulins) and (iii) normal saline made up to 1 c.cm. Regarded as a nutrient medium it consists of one-quarter strength broth, in which already two types of bacteria have been cultivated, and a very minute amount of serum globulin.

Immediately after the cultivation of the staphylococcus from the Bundaberg bottle subcultures were made into other bottles and after varying periods at room temperatures or at 37° C. animals were inoculated. From one bottle doses of 1·0 c.cm. given subcutaneously after one, two, three and nine days at room temperature produced no obvious effects in guinea pigs, but it was noted that whilst the first three animals gained 104, 122 and 92 grammes in weight in twenty-two, twenty-six and twenty-five days respectively, the fourth animal, inoculated with the nine days' growth, only gained seven grammes in a corresponding time.

From another inoculated bottle kept at room temperature, subcutaneous injections of 1·0 c.cm. were given with the following results:

Time After Contamination.	Results in Guinea Pigs.	Weight Before Inoculation.	Weight Afterwards—Days in Parentheses.
4 hours	Nil	249	332 (20)
23 hours	Nil	243	358 (23)
2 days	Late ulceration	250	392 (26)
3 days	Nil	234	324 (26)
4 days	Late ulceration	230	327 (25)
6 days	Firm induration	255	274 (24)
9 days	—	241	242 (21)

The first five animals inoculated on the first four days gained on the average 105 grammes in weight in twenty-four days, whilst the animal inoculated on the sixth day which early showed a firm indurated area in the abdominal wall, only gained 19 grammes in twenty-four days, and the animal injected on the ninth day showed no gain in weight after twenty-one days.

Another series of animals was inoculated subcutaneously with doses from a bottle of Batch 78A (toxin-antitoxin) infected directly from the original Bundaberg bottle and incubated for twenty-four hours at 37° C. One cubic centimetre injected into a guinea pig produced a small local abscess healing to a clean scar by the tenth day and the animal gained only 27 grammes in nineteen days. One-half of a cubic centimetre also produced late ulceration, but the animal gained 67 grammes in nineteen days. One-fifth and one-tenth of a cubic centimetre produced no obvious lesions and the animals gained 63 and 60 grammes respectively in nineteen days. A rabbit showed only a slight local lesion after a subcutaneous inoculation of 0·5 c.cm. and gained well in weight. Four mice received an intraperitoneal inoculation of 0·2 c.cm.. They appeared to be very sick after five hours with neurotoxic symptoms but seemed well on the following day. One died after six days with multiple abscesses in both kidneys.

Another bottle of Batch 78A inoculated direct from the fatal bottle was kept at room temperature. Of two mice inoculated intraperitoneally after twenty-four hours with 0·1 c.cm. one was moribund in four hours and the other survived. After forty-eight hours four mice received the same dose and one only died in four days.

Eleven guinea pigs also were inoculated from two bottles of Batch 78A infected directly from the fatal bottle. Doses ranging from 1·0 to 0·1 c.cm. and injected subcutaneously after varying periods up to four days produced no deaths and scarcely any local effects. The four days' growth, however, produced some necrosis of the skin.

The results of these early animal experiments suggested to us that a more detailed study should be undertaken in which animals would be inoculated daily with the same dose and at the same time the number of cocci enumerated.

Graph 1 shows the counts obtained at intervals in a 10 c.cm. rubber-capped bottle of toxin-antitoxin (Batch 86) marked M.S.C. 4 and also the local effects of subcutaneous inoculation in the abdominal wall of guinea pigs.[1]

The rubber cap was sterilized by filling the cup-like depression in it with methylated spirit, most of which after two minutes was gently shaken out and the remainder flamed. With a sterile one c.cm. tuberculin syringe 0·1 c.cm. of a very dilute saline emulsion of the staphylococcus was injected through the rubber cap and the bottle inverted several times to mix evenly. The same amount was well mixed with melted agar and the latter plated. Counts made after two days at 37° C. show that the inoculum was approximately 550 organisms per cubic centimetre of toxin-antitoxin. This bottle was incubated at 80° F. (26·6° C.) and from the second to the fourteenth day, excepting the tenth and eleventh days, the bottle was taken out on to the bench for several hours, the contents gently agitated and 0·6 c.cm. withdrawn with a syringe. One-tenth of a cubic centimetre was mixed with agar and plated or known dilutions of it were made and plated. At the same time the remaining 0·5 c.cm. was inoculated subcutaneously into a guinea pig. As may be seen from the graph, animals were inoculated after two days and every subsequent day to the fifteenth day inclusive. It is admitted that the withdrawal of the dose for counting by a syringe though graduated in hundredths of a cubic centimetre, is not a very accurate method but it was convenient and suited to the double purpose of this research.

The counting of the plates was done after forty-eight hours and by a different member of the Commission. The highest figure observed, 363 million per cubic centimetre, was reached on the ninth day, though possibly if counts had been done on the eleventh or twelfth days they might have been higher. The fall in the count between the third and fourth days was also noted in another bottle, whilst in a third bottle it was not noted. Otherwise the tendency is towards a steady rise reaching a plateau about the seventh, eighth or ninth day. In the third bottle above mentioned the increase from the end of the first day to the sixth day is roughly in arithmetical progression.

The guinea pig inoculations showed curious results. In Graph 1 it will be seen that 0·5 c.cm. produces marked local infiltrations at two periods, on the sixth day and on the fourteenth and fifteenth days. The admixture of six units of antitoxin did not prevent the reaction on the fifteenth day. There is no obvious correlation with the number of living organisms. The effects of inoculation from this bottle are summarized here.

Days of Incubation.	Results of Inoculations of Guinea Pigs.
2	No infiltration. Weight increased from 201 to 237 grammes in six days.
3	No infiltration. Weight increased from 330 to 378 grammes in five days.
4	No infiltration, but a small lump on second day, disappearing on fifth day. Weight increased from 292 to 304 grammes by fourth day.
5	Slight infiltration (+), developing into a pea-like lump which disappeared by the tenth day. Weight increased from 240 to 265 grammes by the third day and to 287 grammes by the tenth day.
6	In 24 hours an extensive soft longitudinal infiltration (+++) in abdominal wall, more marked after 48 hours, becoming firmer by the fourth day and extending from pubes to ensiform, developing finally into a discrete subcutaneous lump, which did not burst externally, but gradually decreased in size, though still quite large after three weeks. Weight decreased from 264 to 232 grammes by the second day, rising only to 269 grammes by the seventh day.
7	No infiltration, but a very small lump noted on fourth day. Weight increased from 222 to 230 grammes on third day, but was down to 206 grammes on sixth day, rising again to 254 grammes on tenth day.
8	No infiltration, but a small lump noted on fifth day, disappearing promptly. Weight fell from 230 to 217 grammes by fifth day, and then rose.

[1] The graph is not reproduced.

Days of Incubation.	Results of Inoculations of Guinea Pigs.
9	No infiltration. Weight increased from 213 to 223 grammes by fifth day.
10	Slight infiltration (+), followed by a longitudinal band of thickening on third day which localized to a small lump on seventh day. Weight fell from 207 to 147 grammes by third day and rose to 197 grammes by seventh day.
11	No infiltration. Weight about stationary for four days, then rose.
12	No infiltration. Weight fell from 205 to 196 grammes in three days.
13	No infiltration. Weight fell from 210 to 201 grammes in two days.
14	Extensive soft infiltration (+++), with oozing of serum from the needle hole, becoming a firmer longitudinal band after three days, central skin necrosis after four days, then an ulcer which had healed by the sixteenth day. Weight decreased from 212 to 181 grammes in three days, rising to 257 grammes after seven days.
15	Moderately extensive infiltration (++), becoming firmer and band-like after three days, then settling down to form two lumps, one rather deep below costal margin, the other superficial in the lower abdominal wall. The animal was killed on the sixth day, and these lumps were found to be two abscesses connected by a thin band of tissue. No lesions noted in other organs. Weight fell from 210 to 193 grammes in two days.
	Another animal was inoculated with 0·5 c.cm. *plus* 6 units antitoxin. It also developed a definite infiltration (++).

Looking through the weights of animals inoculated with this and other bottles, the main facts elicited are that inoculations after two, three and four days' incubation at Bundaberg temperatures as a rule do not cause loss of weight, inoculations after longer incubations may or may not cause loss of weight, but when the infiltration is severe the loss of weight is usually prompt and definite. Recovery and gain of weight occur after a variable period. The injections from this bottle did not cause death in doses of 0·5 c.cm..

Another rubber-capped bottle inoculated with the Bundaberg staphylococcus gave a somewhat similar sequence of effects. This bottle was kept at room temperature in hot weather, the maximum bench temperature during the eleven days being 93° F., the minimum 65°, whilst the average bench maximum and minimum for the period were 82° and 72° respectively, therefore not far removed from the Bundaberg temperatures (see Appendix 7). The inoculum, counted by plating, amounted to 140 cocci per cubic centimetre of toxin-antitoxin. The results of subcutaneous inoculations of 0·5 c.cm. are tabulated:

Days of Incubation.	Results of Inoculations of Guinea Pigs
2	No infiltration. Weight fell slightly from 295 to 287 grammes in five days, then rose to 313 grammes by tenth day.
3	No infiltration. Weight increased from 255 to 280 grammes in five days and to 295 grammes by ninth day.
4	No infiltration. Weight increased from 244 to 287 grammes in four days, to 303 grammes in six days, then fell to 282 grammes by eighth day.
5	No infiltration, but after three days a small firm lump was noted. It did not ulcerate and gradually grew smaller and was not palpable after seven days. Weight fell slightly from 243 to 237 grammes in five days, still 237 grammes in seven days, and only 247 grammes in nine days.
6	Extensive infiltration (+++) which persisted for several days, then settled down to a firm subcutaneous lump which disappeared by the ninth day. Weight increased from 242 to 286 grammes in four days, fell to 265 on sixth day, and rose to 301 grammes by ninth day. This gain is exceptional.
7	Slight infiltration (+), settling down to a longitudinal band of thickening and later to a firm pea-like lump. Weight fell from 287 to 266 grammes in five days, then rose to 307 grammes by eighth day. The animal was killed by ether on the thirteenth day. The lump proved to be a small well-enclosed abscess containing whitish rather thick pus. Adrenals normal looking. No other lesions found. A film of the pus showed numerous Gram-positive cocci and pus cells and much phagocytosis. A staphylococcus was cultivated.
8	No infiltration, but a very small lump noted on fourth day. It could not be felt on fifth day. Weight fell from 275 to 255 grammes in four days, then rose to 289 grammes by the eighth day.
9	An extensive soft subcutaneous infiltration (+++) and some redness of skin within 24 hours. On third day it was firmer. On fourth day it was incised and bled freely (hæmorrhagic exudate), but no pus was found. Cultures from the incised tissue gave several colonies only of a staphylococcus. The wound began to heal, although the swelling remained. On the tenth day it had resolved into two firm raised lumps. The animal was killed by ether, and dissection showed a superficial abscess in the mid-abdominal wall and a deeper abscess higher up affecting the muscle. The inflammation had caused adhesion of the anterior surface of liver to the abdominal wall, and there were several superficial miliary abscesses in the liver. Staphylococci were cultivated from the abscesses, but the heart's blood was sterile. There was no lesion of the adrenals. The weight had fallen from 294 to 243 grammes in four days, was only 249 grammes after seven days, and 262 grammes after ten days.
10	Slight infiltration (+), settling down to a small lump in three days. This lump was still palpable after ten days, but had disappeared by sixteenth day. The weight increased from 277 to 297 grammes in four days and to 314 grammes in six days.
11	Slight infiltration after 24 hours, but extensive (+++) on second day. A deep incision caused free bleeding, but no pus was found. The wound healed well, but the swelling remained and settled down to a raised lump free from surrounding infiltration by the eleventh day. It then showed fluctuation and settled down partly by discharge and partly by absorption. The weight fell markedly from 261 to 177 grammes in four days, was only 181 grammes on fifth day, and 197 grammes on eleventh day.
12	This animal was given the remaining contents of the bottle, *viz.* 1·4 c.cm. In 24 hours its abdomen showed an extensive œdematous swelling, which oozed serum from the needle hole. On the second day it was much the same, and on the third day the animal looked very ill. It was killed by ether. Dissection showed œdematous subcutaneous tissue and necrotic muscle. The subjacent anterior surface of liver showed some greyish exudate. The organs were somewhat wet. Adrenals not hæmorrhagic, but slightly congested. Staphylococci were cultivated from the heart's blood and from the necrotic tissues. The weight had fallen from 272 to 248 grammes in two days and was 245 grammes after three days.

Sections of the tissues involved in the local lesion of this last guinea pig showed œdema and hæmorrhage in the subcutaneous tissue, degeneration of striped muscle and infiltration of tissues with Gram-positive cocci and leucocytes. Sections of the adrenals showed no abnormality.

In this series the results indicate an initial innocuous period of four or five days' duration, next a short period in which there is a severe local effect, then a period of lessened effect and finally a second active period (possibly more permanent) in which severe local effects are obtained. This bottle gave the highest counts of cocci which have been observed in toxin antitoxin of Batch 86. It was the first bottle to be investigated in this manner. Counts of over 2,000 millions per cubic centimetre were reached, though 400 million was the maximum observed in three other bottles. Possibly the organism in our later experiments had lost some of its ability to grow in toxin-antitoxin. Whether this could be regained by passage through successive bottles of toxin-antitoxin mixture has not been investigated.

Another bottle of Batch 86 inoculated with approximately 700 cocci per cubic centimetre and incubated at 80° F. showed a steady uninterrupted increase in the numbers of living cocci from the second to the sixth day. The results of plating and the effects of subcutaneous inoculations of guinea pigs with 0·5 c.cm. are here detailed.

The remaining contents of this bottle were centrifuged. The organisms deposited from one c.cm. were emulsified in saline and inoculated subcutaneously. No local lesion resulted. Another guinea pig was inoculated with the organisms from 0·5 c.cm. emulsified in saline and mixed with 0·5 c.cm. of the supernatant fluid. On the following day there was definite infiltration which persisted for three days and then settled down to a well-defined lump.

In the series above described, no definite infiltration was produced until the seventh, eighth and ninth days, and the local lesions were not as severe as in the two preceding series. The centrifuged organisms emulsified in saline did not produce infiltration.

(*Continued on page 58.*)

The Medical Journal of Australia

SATURDAY, JULY 14, 1928.

Toxin-Antitoxin Immunization.

THE inquiry conducted by the Royal Commission appointed in connexion with the fatalities that occurred at Bundaberg on January 28, 1928, has raised some very important questions in regard to toxin-antitoxin immunization. In an editorial article published in this journal on February 11, 1928, the main points in the prevention of diphtheria were discussed. At that time the cause of the death of the children at Bundaberg had not been investigated. With the fuller information now available, it may be stated most emphatically that there is no essential or intrinsic danger associated with the injection of the mixture, that this procedure, like every other large undertaking, is rendered unsafe if correct technique is not carried out and that an accident should not be regarded as a reason for the discontinuance of the immunization, provided that it can be shown that it has actually resulted in a substantial diminution of the incidence of diphtheria. It seems, however, that the first contention must be subject to one reservation. According to Professor Birk, of the *Kinderklinik* at Tübingen, it is unwise to immunize adults on account of the severe reactions. At one time all probationer nurses at the clinic were immunized, but the practice was abandoned on this account. The risk to which young women nursing children with diphtheria are exposed, is not very great. The experience at the Metropolitan Asylums Board hospitals teaches that a relatively small proportion of the nurses contract diphtheria and that severe and fatal infections are very uncommon. The reaction to Haffkine's antiplague vaccine is often extremely severe, but the use of this protection against infection is justified on account of the immanence of danger when an adult contracts plague.

Toxin-antitoxin immunization has been employed as a prophylactic measure on a large scale in the United States of America and to a greater or lesser extent in nearly every other country in the world. Dr. W. H. Park in New York, has no doubt concerning the protective value of the injections. He has initiated the campaign in his city and has an experience of well over two million injections. As was pointed out in our previous article, the mortality of the disease has been reduced from 22·7 per 100,000 to 7·9 per 100,000 by its means. These figures have not been challenged. Such excellent results have not been attained in some parts of the Continent of Europe. Some authors have reported that more information is needed before a final decision can be given as to the nature and extent of the immunity conferred on susceptible children by toxin-antitoxin. It is generally held that children who react to the Schick test, are susceptible and that injections of toxin-antitoxin mixture convert these children into non-reactors, which is supposed to indicate that they are immune to diphtheria. It has been shown that some persons react to the Schick test and yet remain uninfected, notwithstanding prolonged exposure to infection. On the other hand, Dr. Park claims that the susceptible children who have been immunized, have escaped infection for a period of ten or eleven years. Again, under rare circumstances non-reactors have contracted diphtheria. It has also been observed that a child has contracted diphtheria shortly after having received injections of the toxin-antitoxin mixture which resulted in the loss of the power to react to the Schick test. These occasional happenings should suffice to place the medical profession on its guard and to stimulate immunologists to determine whether or not the same mechanism is involved in artificially produced insusceptibility to diphtheria, in the non-reactor state and in natural immunity. There is no doubt concerning the protection afforded by toxin-antitoxin injections in the great majority of children. Although the immunity would be of short duration by itself, it certainly becomes lasting when the children come in contact with carriers of diphtheria bacilli or other children affected by the disease. The immunizing effect of epidemics is well known and is suffi-

ciently strong to maintain an immunity once this has been established.

The American health authorities hold the opinion that toxin-antitoxin immunization should be employed as a general measure of prophylaxis and are at present engaged in an immense experiment of endeavouring to immunize every child in New York who reacts to the Schick test. Some Continental authorities, including the British, are opposed to the wholesale application of the measure, at all events until some of the debatable points have been settled. The immunization is recommended for certain child communities and for schools in time of epidemics. There seems to be a substantial objection to enforcement; nearly every European authority claims that better results may be obtained by persuasion. The reason adduced against compulsion is that the voluntary plan necessitates popular education and this leads to a collaboration of the individual with the medical profession and the health authority. In practice, especially during the course of an epidemic, many hygienists dispense with the preliminary cutaneous test and immunize all children without distinguishing between those reputed to be susceptible and those thought to be immune. To sum up, it may be taken that today the application of toxin-antitoxin immunization should be employed sectionally and in chosen children communities, but that no compulsion should be exercised. The medical profession should urge parents to submit their children to this procedure. It is useful in proportion to the extent of its application and therefore it is not to be regarded as essential for single individuals. The risks are known and are avoidable. In Australia great care is taken in the preparation of the mixture. The medical practitioner using the mixture should be certain that contamination does not take place. He can avoid this if he discards mixtures that have become turbid and if he is meticulous in sterilizing the rubber cap of the container and the syringe and needle. The medical profession has the duty at this period of reassuring the public that despite the terrible disasters at Bundaberg toxin-antitoxin immunization is a safe procedure for children and should be carried out when diphtheria is threatening any community.

Current Comment.

NEPHRITIS.

It is impossible to discuss the pathogenesis of nephritis without having a clear conception of the physiology of the normal kidney. The two classical theories of renal excretion are those of Ludwig and Bowman. The fluid which leaves the glomerulus, is a filtrate from the blood; it has the same composition *minus* the colloids. According to Ludwig's theory water is absorbed by the cells lining the tubules as the dilute glomerular filtrate passes over them. Bowman regarded the glomerular function as that of filtration, but also believed that the cells of the tubules secrete the specific contents, such as urea, uric acid *et cetera* and that the filtrate which would be much less copious than required by Ludwig's theory, contained only water and salts. Much work has been done on the subject and the modern view is due mainly to the work of Cushny. It is not necessary to trace the investigations in this place. The conclusions to which they led, have been well described by Bayliss. By the retention of the pure filtration process of the lower animals a filtrate is first made; this filtrate contains all the non-colloidal constituents of the blood in the same concentration as in the latter. If this were to be sufficient to carry away the whole of the waste products which are present in very low concentration in the blood, an enormous loss both of water and valuable constituents would be entailed. To meet this a mechanism has been developed by which not only a great part of the water is reabsorbed, but also a large proportion of the valuable salts such as sodium chloride and also organic foodstuffs such as glucose and amino acids.

The soundest classification of any disease types is that based on a knowledge of the pathological changes which take place in the affected organ from the time that the first deviation from abnormality occurs. At times it is necessary to adopt a clinical classification because the pathology is not completely understood. But even designations based on pathological findings are not always satisfactory. The terms chronic parenchymatous nephritis and chronic interstitial nephritis have been useful and enabled the clinician to visualize the changes which were taking place in the kidneys. At the same time, however, there was the disadvantage that the impression was given that the two conditions were separate entities and not different stages of a progressive lesion. J. Gray has recently carried out some experimental work[1] and has suggested a classification which is in accord with his findings. He refers to the evidence in favour of a bacterio-toxic origin of nephritis and points out that while it is very strong, it falls short of absolute proof. He also shows from the findings of others, that some form of streptococcus is the organism most frequently associated with nephritis. Much of the work on experimental nephritis has been carried out with

[1] *The Journal of Pathology and Bacteriology*, April, 1928.

simple chemical poisons. The effects produced by these means are in some respects fundamentally different from the changes which characterize ordinary acute nephritis. The changes produced by minimal doses of chemical poison are predominently tubular and the glomeruli are affected only as the dosage is raised. Gray believes that when this fact is considered on the basis of the modern theory of renal function, it will be seen that these poisons, being crystalloids and of relatively low molecular weight, are filtered through the glomerular tufts and concentrated in the tubules by reabsorption from the dilute filtrate. A concentration of any considerable degree would in Gray's opinion so definitely overbalance any selectivity for endothelium possessed by any particular poison, that it is reasonable to claim that any other crystalloids used in future would also produce primarily and chiefly tubular effects. Toxins are of high molecular weight and colloids; they are therefore probably retained by the healthy glomerular filter. They are concentrated in the glomerular tufts by the withdrawal of the filtrate and are later rediluted by reabsorption from the filtrate in the intertubular plexus. In other words, the circulating toxins are in a higher concentration in the healthy glomerulus than anywhere else in the body. Gray does not deny that the toxin may pass through the filter after it has caused damage; in fact he relies on this to explain the different phases of damage in acute nephritis.

In his experiments he used diphtheria toxin and the exotoxin of *Streptococcus scarlatinæ*. Rabbits and rats were used, but the former gave more satisfactory results. The animals were killed or died after varying periods. The earliest changes in all cases were endoglomerular. There was very little cellular infiltration, the chief alterations within the glomeruli being destruction of endothelial nuclei followed by necrosis of loops. These were followed by epiglomerular changes, that is, by changes in the epithelium covering the tufts. The changes in these cells were first swelling and then separation. Certain minor differences were noted with the two toxins. Albuminous material did not appear in the capsular space while the epithelium covering the tufts was healthy. If the animal survived this stage, the extension of the damage was indicated by the appearance of albumin and casts in the urine. No distinct tubular changes were found in animals with slight albuminuria. Animals killed at a later stage showed the first signs of tubular degeneration. The distribution of the tubular damage was variable. Usually it was earliest and worst in the loops of Henle, but sometimes the commencements of certain first convoluted tubules were strikingly affected, even in the early stages. The glomerular changes often became intensified for a time and then remained stationary, they sometimes retrogressed to some extent. The pictures thus successively revealed are those of endoglomerular nephritis, endoglomerular and epiglomerular nephritis, glomerulo-tubular nephritis and a nephritis in which tubular changes apparently predominated.

In discussing his findings Gray points out that his observations indicate a sequence sharply demarcated from that of chemical nephritis. After toxin has passed through the damaged epithelium of the tuft, it injures the tubules by concentration through reabsorption. At the later stages the tubular changes become still more prominent relatively to the glomerular, for just in proportion as the tufts become toxin-permeable, so they become less and less the site of a special concentration of toxin; meanwhile the tubules are exposed to a progressively increasing concentration of toxin. Gray sees two difficulties in this reasoning. The first is that acute nephritis in man is described as being glomerular, tubular or glomerulo-tubular. In the second place the occasional cases of acute interstitial nephritis require explanation. In regard to the first of these difficulties he states that it is generally recognized that the names given do not represent sharply defined conditions. The groups shade imperceptibly into one another. He thinks it possible that the different appearances represent not different types of varying causation, but successive phases in a condition of which the mode of onset is uniform. Gray regards acute interstitial nephritis as a condition intermediate between ordinary nephritis and suppurative nephritis in which some multiplication takes place in the kidneys, though it is not sufficiently unrestrained to give the picture of frankly suppurative nephritis. Gray's final classification then includes three groups: The ordinary bacteriotoxic type, the acute interstitial type and the acute suppurative nephritis. The bacterio-toxic type is comprised of the various types of nephritis described in connexion with his experiments.

It will be seen at once that the explanation given by Gray fits extremely well with the present day view of the physiology of the kidney. It is based on a bacterio-toxic origin due to lysis of the organisms in the glomeruli and the release of endotoxins. He has not discussed the causes which might give rise to this supposed lysis. The concentration of the microorganisms in the glomerulus as a result of the filtration process is not sufficient to account for it. If it were, acute nephritis should be an invariable accompaniment of septicæmia. But it is not. Gray states that he has not made any mention of the possible importance in nephritis of sensitization to the toxins and organisms concerned, but that this appears to be a point worth investigating. The more that consideration is given to this subject, the more evident it will become that there must be some susceptibility of the glomerular cells to certain organisms or to their exotoxins. The hydrogen ion concentration of the blood may also have some bearing on the subject. It is in the investigation of primary or essential causes that future workers must busy themselves. This statement is not intended to detract in any way from the work of such investigators as Gray; the structure that he has built, is apparently complete, but it is impossible to estimate it at its true value until the fundamentals have been determined.

Abstracts from Current Medical Literature.

PHYSIOLOGY.

Antidromic Impulses and Herpes Zoster.

FOR several years Lewis and his collaborators have been accumulating evidence tending to show that every form of injury to the skin reaching a certain grade of severity calls forth a series of vascular and nervous responses immediately dependent upon the same fundamental and natural chemical stimulus and that this stimulus is a substance of the histamine type set free in the skin. T. Lewis and H. M. Marvin (*Heart*, 1927, Volume XIV, Number 50) have extended the observations to antidromic impulses and *herpes zoster*. The blistering of the skin in *herpes zoster* is associated with inflammation of posterior root ganglia receiving sensory nerve fibres from the area of skin affected. Evidence excludes centrifugal movement of a poison along the nerve path as an explanation and further herpes of the same type may follow lesions of peripheral sensory nerves. The authors show that antidromic impulses, set up by stimulating sensory nerves, produce the same kind of vasodilatation as does histamine applied locally. They suggest that the local vasodilatation of *herpes zoster* is set up in a similar way. But in *herpes zoster* the skin is not only reddened, it also blisters. They suggest that this is due to the slow development of the condition in *herpes zoster* such as is seen after long exposure to ultra-violet light. *Herpes zoster* is then due to antidromic impulses passing down the sensory nerves and releasing slowly in the skin substances of a histamine-like nature.

Action of Heart Tonics.

R. BODO (*Journal of Physiology*, February, 1928) has studied the effect of various so-called heart tonics on Starling's heart-lung preparation. By heart tone is meant here the mechanical efficiency of the muscle fibre, that is the relation of the mechanical energy to the total energy liberated. If the same work is done by two hearts, in one case with a small diastolic volume and in the other with a large diastolic volume, the mechanical efficiency of the former is greater and the tone of the former is better than that of the latter. The heart tone can then be measured by measuring diastolic volume, provided the work done by the heart is maintained constant during the whole of an experiment. A heart tonic should then enable a heart to perform the same amount of work as before with a smaller volume. Digitalis, caffeine and "Insulin" produce a tonic effect, enabling the heart to expel the same amount of blood with a smaller heart volume. The effect after digitalis is delayed but lasting, after caffeine relatively rapid in onset and evanescent and after "Insulin" it could be observed only once in each preparation. Camphor, sodium nitrite, pituitrin and quinidine produce dilatation of the heart. Strychnine and amyl nitrite have no effect on the tone of the heart. Digitalis, caffeine, camphor and nitrites increase the coronary flow, nitrites and caffeine in a very high degree, digitalis and camphor only slightly. Pituitrin, quinidine and "Insulin" diminish the coronary flow, the two latter only in large doses; in smaller doses these are without effect.

The Respiratory Response to Carbon Dioxide.

AS is well known, when air containing an increased percentage of carbon dioxide is breathed the volume of respiration is increased. That the maximum increase in respiration occurs only after the experiment has continued for some time, does not appear to be definitely recognized and P. Padget (*The American Journal of Physiology*, January, 1928) has studied this aspect of the question. When air containing an increased amount of carbon dioxide is breathed, the maximum increase in respiration occurs after a time which varies as the concentration of carbon dioxide breathed. The reactions of different subjects vary considerably, but in any one subject the same concentration of carbon dioxide in general produces the same effect. Rebreathing experiments done with a small volume of air give too low a response for high concentrations of carbon dioxide, but rebreathing experiments with large volumes of air give results which compare favourably with those obtained by breathing mixtures of carbon dioxide and air. In the case of the small volume the carbon dioxide is of course increasing all the time, but the lag in response prevents the full effect being seen. When carbon dioxide mixtures are inspired, there is no delay in raising the carbon dioxide tension of the arterial blood. From this it appears that the lag in respiratory response may be explained by the time required to saturate the tissues (especially the respiratory centre) to the new carbon dioxide tension.

Energy in Relation to Food Intake.

G. R. COWGILL (*American Journal of Physiology*, May, 1928) has studied the energy factor in relation to food intake in dogs. It is certain that, when all the dietary essentials such as proteins, vitamins and salts are being provided in amounts sufficient to meet the bodily requirements and there are no pronounced psychic influences operating, the energy need becomes the regulator of food intake. The question arises: Does the energy need always govern the urge to eat? Twelve adult dogs ranging from 3·41 to 15 kilograms body weight were fed with all that they wished of a "perfect" diet and the amounts of food eaten were carefully measured. The first reaction to these experimental conditions in every case was complete ingestion of all food offered concomitant with an increase in body weight until a certain weight different for each animal was reached. Thereafter the food intake varied widely from day to day, but the body weight remained remarkably constant. Four dogs were then offered another artificial diet having a much greater energy content. Under these conditions they ate fewer grammes of the ration, but the food eaten represented approximately the same number of calories per day as formerly. Considered in relation to body surface area, the caloric adjustments made by six dogs were approximately the same. In other words, the total metabolism values for the dogs of different sizes were in quite constant relation to their body surface areas. The variations were approximately the same as those shown by the basal metabolism of a normal individual. The author stresses the necessity for giving full accounts of food intake and also the energy value of the ration in all records of feeding experiments.

Pellagra-like Disease in the Dog.

IN 1917 Chittenden and Underhill called attention to the production in dogs of a pathological condition which closely resembles human pellagra. The disease may be induced in dogs by the ingestion of a diet containing boiled peas as the chief source of nitrogen. Under suitable conditions these nutritional disturbances may be manifested even when raw meat is included in the diet. It was found subsequently by other workers that this condition was probably identical with what is called in America "black tongue" in dogs. Further, it has been found that substances which have been found to possess black tongue preventive potency have, when tried, been found to be efficient preventives of human pellagra. The importance of the experimental production of this pathological condition for human nutrition was so obvious that F. P. Underhill and L. B. Mendel (*American Journal of Physiology*, January, 1928) made further extensive investigations. A diet of boiled dried peas, cracker meal and cotton seed oil invariably produced the condition. High nitrogen intake, whether in the form of peas or meat, will not prevent the incidence of the disease, although the symptoms may be somewhat delayed. Yeast will not prevent the disease, but fresh liver will and butter fat contains a factor effective in prevention. When butter fat is fed to dogs suffering from the deficiency disease, the animals are quickly restored to a normal condition. Under similar conditions cod liver oil, known to contain active fat-soluble Vitamin A, failed to restore these diseased dogs. Evidently this vitamin is not the potent factor. The presence of the potent factor in butter fat is associated with the colour of the butter. As the colour

decreases, the potency is diminished. Egg yolk contains the factor, but is apparently less potent than butter fat. Boiled unpeeled carrots are very effective. Crystallized "Carotin" in relatively small doses is capable of curing the diseased condition. These experiments lend support to the conception that naturally occurring pigmentary substances play a rôle in nutritive processes.

BIOLOGICAL CHEMISTRY.

Metabolism in a Test Tube.

E. WATCHORN AND B. E. HOLMES (*Biochemical Journal*, October, 1927) have studied the formation of ammonia and of urea by kidney tissue of the rat grown *in vitro*. The authors had already noted that embryonic tissue of the rat's kidney gave rise to ammonia and urea while growing in the test tube, but that no ammonia nor urea was formed if the tissue remained alive, but did not grow. They were inclined to consider that the combustion of nitrogenous substances supplied the energy necessary for growth. To test this hypothesis the authors have studied the effects of the addition of glucose to the culture medium. Such a substance in their opinion would be a more available source of energy and might be expected to lessen the production of urea and ammonia during the growth of tissue. The authors have found that the addition of glucose not infrequently diminishes the production of urea and ammonia during growth and further that growth may occur without any production of urea or of ammonia. The addition of glucose had no influence on the absence of urea and ammonia when floating (resting) tissue was present.

Diet and Body Fat.

WILLIAM E. ANDERSON AND LAFAYETTE B. MENDEL (*The Journal of Biological Chemistry*, March, 1928) have studied the relation of diet to the quality of fat produced in the animal body. The iodine numbers and refractive indices of the body fat produced in rats on a variety of specially planned dietary *régimes* were determined. In order to ascertain the possible changes in the character of the fat deposited at various stages of development, observations were made on animals receiving a uniform mixture of whole wheat and whole milk. On this ration the body fat tends to have a lower iodine value, that is, it becomes harder as the body weight increases. A scheme of experimental feeding was devised by which the animals received 15% of their total calorie intake in the form of protein. This was supplied by dried skimmed milk in amounts to furnish 40% of the total calories of the ration. The remaining 60% was variously offered in the form of fat or carbohydrate. The nutrients available for fat formation could thus be varied at will. When soy bean oil, corn oil, cotton seed oil or peanut oil was ingested, the resulting body fat resembled the food fat. On the other hand, the body fats produced when butter fat or coconut oil was consumed, contained considerably more unsaturated fatty acids than the ingested fat. When the carbohydrates, corn starch, dextrin and dextrimaltose were substituted in a number of calories equal to those of the fat in the diet, "hard" body fats with iodine values of about 60 were obtained. When proteins (casein and cotton seed globulin) were fed in exceptionally high concentration, amounting to 96% of the total calories, a "harder" fat was produced, approximating in its iodine value the fat of the rats receiving the diets rich in carbohydrates. When a particular type of "soft" body fat has been developed, its chemical make-up can be altered by a change to the hardening ration rich in carbohydrate. Such alterations which presumably involve a partial depletion or transformation of the fat reserves, are accomplished only at a slow rate. The change from one type of specially produced fat to another can be brought about far more rapidly by depleting the fat reserves through brief periods of fasting prior to the change in the dietary.

Pigeons on Rice Diet.

R. R. WILLIAMS (*Biochemical Journal*, October, 1927) brings forward evidence which he considers to suggest that there is present in the outer coats of rice a variable amount of toxic substance. He thinks that further study is needed to determine whether some "deficiency diseases" may not be due to toxæmias consequent on or incident to dietary disturbances in animals fed largely on a single food. The evidence of the author is based on the results of feeding pigeons on rice bran, rice meal and rice polish. These are produced in a rice mill in the proportions of fourteen of bran, ten of meal and three of polish. Six kinds of experiments have been completed. In the first type pigeons have been given polished rice *ad libitum*. Each pigeon has also been given either three grammes bran, three grammes meal or three grammes of polish or the same quantity of a mixture of these substances in the proportions in which they are yielded in a mill. All the pigeons have remained well for sixty days. In the second type of experiment pigeons have been fed on rice bran. The birds have died about the thirtieth day, having lost about 40% of their weight. Examination of the sciatic nerves by Marchi's method revealed degeneration. In the third group of experiments pigeons have been given rice meal. All were well after one hundred and twenty days and no changes were found in the sciatic nerves. In the fourth kind of experiment pigeons have been fed on rice polish. All the birds lived one hundred and twenty days, though they lost weight at first. About half the birds showed moderate degeneration of the sciatic nerves. In the fifth type of experiment the pigeons have been given the mixed rice by-products. About half the birds lost weight steadily until 66% of weight had been lost in from seventy days to one hundred and twenty days. Some pigeons survived one hundred and twenty days. The other pigeons maintained weight and strength. No degeneration of the sciatic nerves was observed. In the sixth group of experiments another sample of rice polish has been fed to pigeons. All died in from ten to thirty-six days. Degeneration was present in the sciatic nerves, but only slightly in the pigeons dying in ten to fifteen days.

Urinary Albumin.

GENERALLY albuminuria is accompanied by a decrease of albumin in the blood and L. F. Hewitt (*Biochemical Journal*, Volume XXI, Number 5, 1927) has investigated the question as to whether blood albumin passes through the kidney unchanged or whether there is a difference between blood and urinary albumin. His previous work has shown that purified proteins possess a characteristic optical rotatory power and dispersion by which they may be identified. In this investigation he found that purified albumin obtained from the urine of patients with chronic nephritis or those with albuminuria of pregnancy possessed the same optical rotatory power and dispersion as serum-albumin; the two are therefore probably identical.

Andrewes's Diazo Test.

G. A. HARRISON AND R. J. BROMFIELD (*Biochemical Journal*, Volume XXII, Number 1, 1928) have failed to confirm Hewitt's hypothesis that the reaction in Andrewes's diazo test is due to a cyclic amine such as tyramine or histamine and they have investigated the solubilities and other properties of "X," the unknown substance; all attempts to make even a rough separation of "X" have proved unsuccessful. The authors refer to the fact that a reaction has been obtained from alcoholic extracts of normal urine which also gave a positive result with the indican test, and indicanæmia is known to occur in uræmia. They found that all specimens of serum giving a positive result with Andrewes's test also gave a reaction to the Jaffé test for indican and that N-acetylindoxyl and potassium indoxyl sulphate both gave a typical Andrewes's reaction in alcoholic or aqueous solutions and solutions of these substances in normal human serum containing one milligramme per hundred cubic centimetres or less also gave a positive result to the test. The authors conclude that the substance in the serum of uræmic patients responsible for Andrewes's reaction is an indoxyl compound, presumably potassium indoxyl sulphate or possibly in part indoxyl glycuronate.

(*Continued from page 52.*)

Days of Incubation.	Numbers of Cocci in Millions (Plate Counts).	Results of Inoculations of Guinea Pigs.
1 (21 hours)	4½ ..	Not inoculated.
2 (51 hours)	92.. ..	Doubtful slight infiltration next day, but nothing on second day. (Either trace or 0.) Weight increased from 269 to 300 grammes in three days.
3	130.. ..	No infiltration. Weight remained about stationary during subsequent nine days.
4	154.. ..	No infiltration. Weight increased from 220 to 289 grammes in three days, then fell to 258 grammes by the sixth day and to 254 grammes by the eighth day.
5	173.. ..	No infiltration. Weight increased from 258 to 287 grammes in two days, then fell to 265 grammes by the seventh day.
6	231.. ..	No infiltration, but a very small thickened area was noted after three days and four days, but was gone by the fifth day (tr.). Weight fell from 227 to 215 grammes in three days, then rose to original level by fifth day and to 247 grammes by seventh day.
7	Not counted	A definite but slight infiltration (+) on the next day, not increased on second day. On the third day it was noted as a longitudinal band of thickening which became firmer, then a broken patch of skin with thickening beneath which was found discharging pus on the seventh day and was almost healed by the tenth day. The weight fell from 200 to 144 grammes in three days, was 167 grammes on fifth day and 207 grammes on seventh day.
8	Not counted	Extensive infiltration (++), which was felt from pubis to ensiform, but was less on second and third days. A longitudinal band of thickening was noted on fourth day continuing until seventh day. By the tenth day it had gone on to ulceration and discharge, healing completely later. The weight fell from 212 to 165 grammes in two days, was 165 grammes on fourth day, 177 grammes on sixth day, and 225 grammes on tenth day.

Another series was carried through with the inoculated bottle in an incubator set at 37° C.. The inoculum was approximately 1,000 cocci as determined by plating. There was slight turbidity within eighteen hours. The results were as follows:

Days of Incubation.	Results of Inoculations of Guinea Pigs.
1 (22 hours)	Definite infiltration (between + and ++) next day, not increased on second day, settling down to a small lump which was palpable until the seventh day. The weight decreased from 201 to 159 grammes in three days, was 183 grammes on fifth day and 194 grammes on twelfth day.
2	Doubtful slight infiltration (tr.). The weight increased from 190 to 210 grammes in two days to 228 grammes in four days and to 256 grammes in six days.
3	No infiltration. The weight fell from 207 to 179 grammes next day and rose to 208 grammes by third day.
4	No infiltration. The weight was stationary for two days.
5	Very slight infiltration 24 hours, but more definite next day (+), and persisted until fourth day, after which it settled down without forming any lump. The weight fell from 204 to 181 grammes in three days, then rose.
6	No infiltration. The weight increased from 185 to 207 grammes in two days, but was only 204 grammes on seventh day.
7	No infiltration. The weight fell from 217 to 212 grammes next day, but rose to 244 grammes by sixth day.
8	No infiltration. Weight was 197 grammes on day of inoculation and only 206 grammes six days later.

The counts obtained in this series at 37° C. were surprisingly low and irregularly variable from day to day, the maximum count being 129 millions per cubic centimetre. Infiltrations were obtained with the toxin-antitoxin after twenty-two hours' incubation and lesser reactions after two and five days, but not after three, four, seven eight and nine days.

To another bottle was added half the percentage amount of iodine present in the remains of Dr. Thomson's bottle (Appendix 19) (the concentration found was probably a maximum reached gradually during the series of inoculations at Bundaberg). The actual amount of iodine added to the 10 c.cm. bottle was 0·5 c.cm. of a solution containing 0·4 mgm. per cubic centimetre. The maximal count obtained in this bottle, incubated at 80° F., was 200 millions per cubic centimetre, so that there was no definite evidence of any inhibitory effect on growth with this concentration of iodine. Guinea pigs were inoculated every day from the third to the fourteenth day, excepting the fifth, eighth and twelfth days. No infiltrations resulted, but the inoculations on the seventh day produced a late and very small ill-defined superficial lump, and that on the tenth day produced a somewhat larger lump first noted on the second day and persisting until on the eighth day it was found discharging a little.

It is unfortunate that inoculations were not done on the sixth and eighth days, and considering this omission we are not justified in assuming that the presence of this amount of iodine inhibits the production of the local lesions in guinea pigs.

Other investigations were carried out on the growth of the Bundaberg staphylococcus in an antitoxin dilution of the same strength as in toxin-antitoxin mixture, the toxin being replaced by nutrient broth diluted one in five (Appendix 25). Counts were made at intervals and the figures obtained were higher than in most bottles of toxin-antitoxin. The results were as follows:

Day.	Numbers of Cocci (by Plating).
2	144 millions
3	227 millions
4	443 millions
5	403 millions
6	636 millions
7	560 millions
8	—
9	694 millions
10	482 millions

A few guinea pigs were inoculated from this bottle and two others of the same preparation, but unfortunately inoculations were not carried out during the first eight days. No reactions were obtained on the ninth and tenth days; on the eleventh day one bottle gave a definite infiltration (++) and the other two did not, and on the twelfth day the former did not, whilst one of the others produced a definite infiltration (+). On the thirteenth day neither of these produced any reaction.

Similarly a dilution of diphtheria toxin was made up of the same strength as in toxin-antitoxin, antitoxin being replaced by 1 in 1,350 horse serum. The same inoculum was introduced as was used above for the antitoxin and a few counts were made. The figures obtained were:

Day.	Numbers of Cocci (by Plating).
2	172 millions
3	170 millions
4	301 millions
5	242 millions

Obviously it was not possible to investigate staphylococcal reactions in animals without first destroying the diphtheria toxin and there seemed to be no useful purpose to be pursued along these lines. The figures are given here for comparison with growth in antitoxin (Appendix 25).

It may be contended that the variations in the reactions of individual guinea pigs described above are only the expression of variation of susceptibility in the animals themselves. We did not have sufficient animal accommodation at our disposal to enable us to inoculate several animals each day from each bottle of toxin-antitoxin mixture, as we were using many guinea pigs daily for the titration of diphtheria toxin and antitoxin. We have been able to show, however, that while individual variations

in the susceptibility of guinea pigs do occur, they are insufficient to account for our results.

In young guinea pigs of about 200 grammes weight which have been used in all the later experiments, variations have been found in groups of three guinea pigs inoculated from two bottles of toxin-antitoxin on different days. Increasing degrees of reaction are expressed as 0 (no reaction), tr, +, ++ and +++.

Group 1	0, 0 and +
Group 2	0, tr and +
Group 3	Tr, tr and tr
Group 4	Tr, tr and +
Group 5	+, + and +
Group 6	+, + and ++
Group 7	+, + and ++
Group 8	+, ++ and +++

We now attempted to ascertain whether when the staphylococcus was grown in richer media in the absence of toxin and antitoxin the same phenomena could be produced. For this purpose we used tubes containing 10 c.cm. of broth inoculated with one loopful of standard emulsion (approximately 1,000 millions per cubic centimetre). After growth for various times at Bundaberg temperature the broth was well shaken up and most of it filtered through Seitz filters, using discs through which bacteria could not penetrate. The filtrate and the unfiltered material were injected subcutaneously into different guinea pigs.

In no case between the twelfth hour and the eighth day did filtrate from any of the cultures in doses of 2 c.cm. cause any lesion, though after thirty and forty hours' growth the injection caused some slight loss of weight.

The unfiltered cultures in a dose of 0·5 c.cm. produced the following results:

Age of Culture.	Local Lesion.	Remarks.
12 hours	Nil	No loss of weight.
19 hours	Nil	Slight loss of weight.
23 hours	Extensive soft infiltration (+++), followed by necrosis of skin on third day	Regaining weight by seventh day.
28 hours	++ Infiltration. Ulceration and discharging abscess by third day	Weight regained by sixth day.
30 hours	++ Infiltration	Weight regained by eighth day.
40 hours	++ Infiltration	Weight regained by fourth day.
3 days	+++ Infiltration. Necrosis by second day	Weight regained by fourth day.
4 days	++ Infiltration	Weight regained by sixth day.
6 days	+++ Infiltration	Weight regained by sixth day.

As the filtrates were in all cases innocuous even in large doses, we next studied the effect of adding increasing numbers of organisms to filtrates and to sterile broth which were injected subcutaneously. One-half of a cubic centimetre of standard emulsion, equivalent to 500 million organisms, with 0·5 c.cm. and 2·0 c.cm. of unfiltered sterile broth gave infiltrations in all respects similar to those recorded with unfiltered broth cultures. One-tenth of a cubic centimetre of broth *plus* 0·5 c.cm. of standard emulsion gave only a trace of infiltration and similar but less marked reactions were produced with 0·5 c.cm. standard emulsion and 0·5 c.cm. of filtrate from 24 hours' broth culture. Standard emulsion alone in doses from 0·5 to 1·0 c.cm. never produces any trace of infiltration.

We next attempted to reproduce the phenomenon by emulsifying the organisms off an agar slope eighteen hours old, in three c.cm. toxin-antitoxin mixture. This emulsion was estimated to contain very approximately 16,000 million staphylococci per cubic centimetre. A series of dilutions were made of this emulsion using toxin-antitoxin mixture as a diluent and 0·5 c.cm. of each was injected into pairs of guinea pigs of about 200 grammes weight. The following results were obtained, the degree of infiltration being indicated as heretofore:

Number of organisms (in millions) injected.	Local lesion after 16 hrs.	40 hrs.	64 hrs.
8,000	++++	+++	+++
	++++	+++	++
4,000	+++	+++	+++
	+++	+++	+++
2,000	+++	++	++
	+++	++	++
1,000	+++	++	++
	+++	+++	++
500	++	Found dead	
	++	+	+
250	tr	tr	tr
	++	++	++
125	+	++	++
	+	++	++
62	+	0	0
	0	0	0

While our plate counts in the experiments with toxin-antitoxin mixture inoculated with staphylococci indicate that the number of living organisms is not the explanation of the phenomenon we have described, it may well be that the total number of living and dead organisms is the determining factor in the production of infiltration.

Is Toxin Liberated from Toxin-antitoxin Mixture by the Staphylococcus?

It might be thought that the lesions we have described in guinea pigs were due to diphtheria toxin liberated from toxin-antitoxin mixture by the activity of the staphylococcus. It is quite certain that this is not the case since broth cultures of the staphylococcus, and cultures in antitoxin alone, produce similar lesions. Further, the infiltrations produced by subcutaneous injection cannot be prevented by the simultaneous administration of large doses of antitoxin. Toxin-antitoxin mixture in which staphylococci have been grown has at no time produced intradermal skin reactions in guinea pigs when injected in doses of 0·2 c.cm. No late paralyses have followed in any of the guinea pigs injected subcutaneously with doses of 0·5 to 1·0 c.cm. and finally the microscopic examination of the infiltrations has shown them to resemble cellulitis of staphylococcal origin. The large series of experiments which we have made with staphylococci growing in toxin-antitoxin mixture under various temperature conditions and the experiments described in Appendix 21 with *B. proteus* definitely exclude the possibility that toxin can be liberated by the action of proteolytic organisms and in particular by the Bundaberg staphylococcus.

Experiments with Monkeys.—We did some further experiments with monkeys which we hoped would prove to be more susceptible to the Bundaberg organism than other laboratory animals. Unfortunately this was not the case, but the general results obtained by the subcutaneous injection of toxin-antitoxin mixture injected with the staphylococcus and incubated at 73° to 85° F. for varying periods, were on the whole confirmatory of our observations on guinea pigs. Our first experiments were carried out on the same three monkeys which had been already used for testing the contents of Dr. Thomson's bottle. They had then shown no ill effects. One of these animals on each day received a dose of 1·0 c.cm. from each of two bottles infected with the staphylococcus, in the right thigh from one and in the left from the other bottle.

Monkey 2 who received his inoculations on the third day of incubation, remained perfectly well, showed no rise of temperature and developed no local lesion at the site of inoculation.

Monkey 1 who received his inoculations on the fourth day, had slight pyrexia on the first and second days after inoculation. His general condition was poor for several days and within twenty-four hours of the injection there was a firm swelling in the left groin which appeared to consist of swollen glands; by the fourth day a definite abscess appeared to be forming. By the second day a slight firmness was apparent in the adductors and hamstrings in the right thigh. This gradually developed and formed a very large abscess which with that on the left side was opened on the tenth day and a quantity of pus was evacuated.

Monkey 3 who received his inoculations on the fifth day of incubation, did not show any pyrexia following his injections but was definitely sick for two or three days. On the third day a painful lump appeared in the right groin. This had cleared up by the sixth day when an area of hard infiltration was observed for the first time in the adductor muscles of the right thigh. By the ninth day this had cleared up and the monkey remained perfectly well.

Further experiments were made on much younger animals which had not had any previous injection.

Monkey 5, weighing 2·1 kilograms, received in the right thigh a subcutaneous injection of 0·5 c.cm. of toxin-antitoxin mixture infected with staphylococci and incubated fifty-five hours, and in the left thigh a similar dose of material, incubated thirty-one hours. The animal's temperature was slightly elevated (39·6° C.) on the first day, though there were no marked general symptoms. On the second day there was a small hard lump on the left side at the site of the injection, but this disappeared by the fifth day. On the right side there was no definite lesion except some firmness in the adductor region on the second day.

Monkey 6, weighing 2·27 kilograms, received in the right thigh a subcutaneous injection of 0·5 c.cm. of toxin-antitoxin infected with the staphylococcus and incubated for three days at room temperature. In the left thigh a similar dose of material incubated for two days was given. There was no definite general reaction, but a firm infiltration of the adductor muscles on the right side appeared on the first day and persisted to the twelfth day. On the left side a small subcutaneous lump developed and persisted to the fifth day.

Monkey 7, weighing 2·35 kilograms, received in the right thigh a subcutaneous injection of 0·5 c.cm. of similar infected material incubated four days and in the left thigh material incubated for three days. On both sides a firm subcutaneous lump appeared associated with some induration of the adductor muscles. These lesions had disappeared by the ninth day.

Monkey 8, weighing 3·11 kilograms, received similar doses of material incubated four and five days respectively. No local lesions resulted and there were no general symptoms.

Monkey 9, weighing 1·42 kilograms, was inoculated subcutaneously in each thigh with 0·5 c.cm. of toxin-antitoxin mixture infected with the Bundaberg staphylococcus, incubated for four days at room temperature and not disturbed in any way till one hour before it was used. (An attempt was thus made to imitate the conditions in Dr. Thomson's bottle.) The general reaction following these injections was very slight and the local lesions were definite but transitory.

Twenty-five minutes after the subcutaneous inoculations approximately 4·0 c.cm. of blood was withdrawn from the heart with a syringe and the blood distributed in graded quantities both into broth tubes and into melted agar, the latter being poured into Petri dishes. No staphylococci were cultivated from any of these fractions of the 4·0 c.cm. of blood. In a previous experiment with another monkey no staphylococci were cultivated from approximately 3·0 c.cm. of heart blood one and a half hours after a subcutaneous inoculation.

Twelve days after the inoculation this monkey went off his food and became quiet and listless. On the fourteenth day it weighed only 1 kilogram. It had some diarrhœa, refused food and was moribund on the sixteenth day. After death by chloroform an autopsy was performed. There were no abscesses in the thighs or in the internal organs. The heart blood was sterile. The liver showed numerous greyish-white flecks, of irregular size, under the cupola, but on incision no pus was found and cultures from them remained sterile. Microscopical examinations of sections showed a heavy coccidial infection. The spleen and kidneys showed no lesion. The mucous membrane of the colon was slightly swollen and of a light pink colour but without macroscopic ulceration. The mesenteric lymph glands were markedly swollen and firm and only colon bacilli were cultivated from them.

These experiments, though carried out upon relatively insusceptible animals, suggest that at temperatures approximating to those at Bundaberg, there is an optimum time for the development of pathogenicity of toxin-antitoxin mixture infected with the staphylococcus.

Generalization of staphylococcal infection cannot be produced by the subcutaneous route in *Macacus rhesus*.

APPENDIX 27.

On the Development of Turbidity in Toxin-antitoxin Mixture in Relation to the Number of Staphylococci Present in Dr. Thomson's Bottle on January 27, 1928.

The Bundaberg staphylococcus forms a "smooth" growth both in broth and in toxin-antitoxin. A slight deposit appeared at the bottom of the amber coloured rubber-capped bottles in which the toxin-antitoxin was kept during the experiments recorded in Appendix 26, but the haziness or turbidity in the supernatant liquid was usually free from visible particles. To detect very slight haziness it is necessary to examine the amber bottles side by side with a sterile bottle. Dr. Thomson stated in evidence before us that on January 28 (the day after the inoculations) he noted that the contents of the bottle were turbid though on the afternoon of January 27 (when he made the inoculations) it had been clear. He had in his possession only one bottle of toxin-antitoxin and, therefore, had no standard of comparison. From a considerable experience of turbidity in these bottles and from the very strong probability that the contamination took place not later than January 24, we consider that some degree of turbidity was present on January 27. The time in which turbidity appears depends on the number of cocci injected through the rubber cap into the bottle, upon the temperature of incubation and possibly upon other factors which we have not elucidated. We endeavoured to obtain a uniform inoculum using a known dilution of a saline emulsion made with a platinum loop from an eighteen to twenty-four hours' growth on agar. The number of living organisms was controlled by plating.

At temperatures between 75° and 85° F. (24° to 29° C.) slight turbidity is visible in from thirty-two to forty-eight hours with a very small inoculum, *e.g.* in two experiments in which agar plate counts showed the number inoculated to be 500 and 700 cocci respectively per cubic centimetre of toxin-antitoxin. At 37° C. the turbidity appears more rapidly, *e.g.* in one experiment an inoculum of 1,000 cocci per cubic centimetre determined by plate culture produced slight turbidity within eighteen hours. The turbidity increases, but never becomes as great as in nutrient broth, nor did we in an experiment in which the bottle was incubated at 37° C. find any greater turbidity. There is a definite lag in growth under the conditions of these experiments. For example, in an experiment at room temperature (75° to 85° F.) with an inoculum of approximately 140 cocci, plate counts after four hours gave 100 cocci per cubic centimetre, after eight hours 450, after twenty-eight hours approximately 3,000,000 and after forty-six hours 29,000,000. In another experiment at 80° F. an inoculum of 730 cocci per cubic centimetre had increased to 4,500,000 in twenty-one hours.

Even allowing that Dr. Thomson's observations were correct, *viz.* that the bottle was clear on January 27 and turbid on January 28, our experiments indicate that the number of cocci present on January 27 would have been, on an extremely conservative estimate, not less than 1,000,000 per cubic centimetre. For example, in the experiment with a very small inoculum only 100 cocci per cubic centimetre were found after four hours, but after forty-six hours when very slight turbidity was noted the number of cocci was 29,000,000 per cubic centimetre. On the day before turbidity was noted, the count showed 3,000,000. Again, in the experiment with a larger inoculum, enumerated as 730 coccci per cubic centimetre, the count on the day on which turbidity was first noted (but not less than six hours after it appeared) was 92,000,000, and on the day before the count showed 4,500,000 per cubic centimetre. Further, in another experiment where the count was made in the afternoon of the day on which turbidity was first noted, the number was found on this day to be 116,000,000, but it was not counted on the day before. These experiments were either at room temperature (82° to 72° F.) or in an incubator set at 80° F.

But if, as we hold, the contamination of the bottle took place not later than the day on which it had been previously used, *i.e.* not later than January 24, then from our experiments it would have been slightly turbid on January 26, the turbidity depending on the factors enumerated and probably more turbid on January 27. Our counts after three days, using small but variable inocula, are in the same three experiments respectively 264, 130 and 265 millions per cubic centimetre.

To obtain these small numbers of cocci we took a fresh saline emulsion which from its opacity we judged would work out somewhere in the neighbourhood of 1,000 million per cubic centimetre. One-tenth of a cubic centimetre was added to 9·9 c.cm. of saline and well mixed, and of this 0·1 c.cm. was added to a further 9·9 c.cm. of saline. Either 0·1 c.cm. of this dilution was used as the inoculum or 0·1 c.cm. of a further tenfold dilution. If, as in one experiment, 1,400 colonies were obtained by plating 0·1 c.cm. of the latter, then the inoculum per cubic centimetre of toxin-antitoxin in the 10 c.cm. bottle was 140 cocci, and the strength of the original emulsion approximately 1,400,000,000 per cubic centimetre. Now we do not know how many cocci were admitted to Dr. Thomson's bottle and it would be unjust to presume that it was above the minimum that would succeed in multiplying sufficiently to cause turbidity. The possibly longer lag in this case and the small initial number of organisms might well have insured smaller numbers, if counts had been made, than we have obtained. Our smallest estimate after three days at the specified temperatures was 130,000,000 per cubic centimetre. Dividing this by three, as we have done in the consideration of our first hypothesis (on which we presumed Dr. Thomson to have been correct in his observations as to turbidity), we find on this second hypothesis (on which we presume that the bottle was contaminated on January 24) that the bottle would have contained not less than 40,000,000 per cubic centimetre on January 27. This again is an extremely conservative estimate.

If Dr. Thomson had contaminated the bottle only during the final inoculations on January 27, then with a very small inoculum it would not have become turbid on January 28, when he noted it, unless the inoculum had been a heavy one, a contingency which we consider extremely unlikely. Contamination on January 27, moreover, would not explain the abscess formation in the arms of all the children who survived.

A further observation was made on the turbidity of toxin-antitoxin in amber bottles. An addition of Bundaberg staphylococci to toxin-antitoxin so that it contained not less than 8,000,000 per cubic centimetre could not be detected by two of us. A concentration of not less than 12,000,000 could just be detected by two of us by careful observation and comparison with a control bottle in a suitable light. A concentration of not less than 20,000,000 could be detected by a third trained observer without previous practice with these bottles. But even a concentration up to 80,000,000 per cubic centimetre does not cause gross turbidity and might easily be missed if the contents were not closely observed.

APPENDIX 28.

Investigation of Content of Diphtheria Antitoxin in the Sera of the Survivors of the Injection of Toxin-Antitoxin Mixture on January 27 at Bundaberg.

For this purpose two methods were used, (i) the protection of guinea pigs of approximately 250 grammes weight against the subcutaneous injection of the certainly lethal dose (C.L.D.) of a sample of diphtheria toxin by its mixture with varying doses of the sera to be tested and (ii) the intradermal method first described by Romer and his colleagues (1909).

The toxin used, pooled 21A and 21D of the Commonwealth Serum Laboratories, was similar to that used in the manufacture of Batch 86. 21A was planted on October 12, 1926, with the C.S.L. strain of Park 8 and 21D with R.G.W. from the Lister Institute. 21A was collected October 20, 1926, and 21D on the following day. They were later pooled. This toxin, approximately seventeen months old, was used in all our experiments. It had an L+ dose of 0·225 c.cm. when tested at the Commonwealth Serum Laboratories on December 13, 1927, but when tested by us in March, the L+ dose was found to be approximately 0·250 c.cm. We did not accurately determine its M.L.D., but this evidently was not far removed from its C.L.D. The L+ dose for this toxin probably does not contain more than 30 M.L.D.'s.

For the use of the first method we determined the C.L.D. of the toxin used. This, as will be seen from Table I, was 0·01 c.cm.

TABLE I.
DETERMINATION OF C.L.D. FOR TOXIN 21A AND 21D.

Number of Animals Tested.	Dose—Toxin.	Result.
4	0·007 c.cm.	Two died on the seventh and tenth days; two survived.
5	0·008 c.cm.	Died on the third, eighth, thirteenth and twenty-fourth days; one survived.
4	0·009 c.cm.	Died on the fifth, fifth and twelfth days; one survived.
6	0·01 c.cm.	Died 40 to 69 hours.
4	0·012 c.cm.	Died 41 to 72 hours.
4	0·014 c.cm.	Died 31 to 56 hours.
4	0·016 c.cm.	Died 28 to 72 hours.
4	0·018 c.cm.	Died 31 to 46 hours.
4	0·020 c.cm.	Died 31 to 53 hours.
4	0·04 c.cm.	Died 31 to 46 hours.

We next determined the amount of standard antitoxin necessary to insure survival when injected subcutaneously together with 0·01 c.cm. of toxin. In all cases the mixtures were allowed to stand at room temperature for one hour before injection. Table II shows the protective effect of varying doses of standard antitoxin. The standard antitoxin used throughout was prepared by the United States Public Health Service, Hygienic Laboratory, Washington.

TABLE II.
PROTECTION AGAINST THE C.L.D. (0·01 C.CM.) OF DIPHTHERIA TOXIN WITH VARYING DOSES OF STANDARD ANTITOXIN.

Dose of Antitoxin.	Number of Animals Tested.	Results.
$^1/_{200}$ unit	2	Died on third and fifth days.
$^1/_{100}$ unit	2	Died on fourth and tenth days.
$^1/_{70}$ unit	4	Two died on third and fourth days; two showed infiltration and necrosis, regaining weight by fourteenth day—definite weakness of hind limbs on twenty-first day.
$^1/_{60}$ unit	4	All showed infiltration and necrosis; regaining weight by tenth day.
$^1/_{50}$ unit	6	All showed infiltration and necrosis; two lost weight, but commenced to regain it on seventh day.
$^1/_{40}$ unit	3	All showed infiltration and necrosis; two loss of weight, regained by thirteenth day.
$^1/_{30}$ unit	4	All showed infiltration and slight necrosis; weight regained by the sixth, tenth and thirteenth days.
$^1/_{25}$ unit	2	No infiltration; steady gain of weight.

The dose of antitoxin necessary to insure survival was $^1/_{60}$ unit antitoxin, while that which gave complete protection (freedom from infiltration and loss of weight) was $^1/_{25}$ unit.

We were now able from the protective effect of varying doses of the sera to deduce the amount of antitoxin present in them.

For the intradermal method we used the technique described by Glenny and Allen (1921), taking as a standard the reaction produced by the injection of that amount of toxin ($^{Ln}/_{500}$) which together with $^1/_{500}$ unit of standard antitoxin just suffices to produce necrosis. The sera were put up each in a series of dilutions mixed with this dose of toxin and the total volume injected was in each case 0·2 c.cm. Mixtures were allowed to stand one hour before injection. Albino guinea pigs were used. The flanks were shaved and four intradermal injections were made on each side, without anæsthesia. In every animal a standardizing injection of the $^{Ln}/_{500}$ of toxin mixed with $^1/_{500}$ standard antitoxin was made and proved of value as there was some individual variation in the sensitiveness of different animals.

Determination of the $^{Ln}/_{500}$ Dose.

Several experiments were made, the range of doses used being as follows: 0·0004, 0·0005, 0·0006, 0·0007, 0·0008, 0·0009, 0·001, 0·0012, 0·0014, 0·0016, 0·0018 and 0·002 c.cm. of toxin. The smallest dose which with 0·002 c.cm. standard antitoxin produced definite necrosis in different animals was either 0·0008 or 0·0009 c.cm. We therefore selected the latter as the $^{Ln}/_{500}$ dose.

As a test of our ability to obtain satisfactory results by these methods, we examined the sera of a number of children in Melbourne who had previously been Schick tested and some who had later been partially immunized and retested. The results obtained are shown in Table III and the details of the protection tests are shown in Table IV.

TABLE III.

RESULT OF TITRATIONS OF ANTITOXIN IN SERA OF CHILDREN WHO HAD BEEN SCHICK TESTED.

		Age.	Intradermal Tests.	Protection Experiments.
			Schick Negatives.	
1	R.G.	3	1 c.cm. contains about $^{1}/_{5}$ unit AT.	1 c.cm. contains about $^{1}/_{5}$ unit AT.
2	H.T.	3½	1 c.cm. contains less than $^{1}/_{10}$ unit AT.	1 c.cm. contains about $^{1}/_{42}$ unit AT.
3	L.A.	6	1 c.cm. contains between $^{1}/_{10}$ and $^{1}/_{40}$ unit AT.	1 c.cm. contains between $^{1}/_{18}$ and $^{1}/_{34}$ unit AT.
4	G.B.	7	1 c.cm. contains about $^{1}/_{5}$ unit AT.	1 c.cm. contains about $^{1}/_{5}$ unit AT.
5	L.A.	7	1 c.cm. contains about $^{1}/_{5}$ unit AT.	1 c.cm. contains less than $^{1}/_{5}$ unit AT.
6	D.R.	8	1 c.cm. contains about $^{1}/_{5}$ unit AT.	1 c.cm. contains about $^{1}/_{5}$ unit AT.
			Schick Positive.	
7	E.R.	2½	1 c.cm. contains less than $^{1}/_{40}$ unit AT.	1 c.cm. contains less than $^{1}/_{60}$ unit AT.
8	J.C.	3	1 c.cm. contains less than $^{1}/_{40}$ unit AT.	1 c.cm. contains less than $^{1}/_{42}$ unit AT.
9	V.M.	4	1 c.cm. contains less than $^{1}/_{30}$ unit AT.	1 c.cm. contains less than $^{1}/_{60}$ unit AT.
			Previously Schick Positive but Immunized nearly Three Months before Sera Tested	
10	B.H.	4	Now Schick positive. 1 c.cm. contains less than $^{1}/_{40}$ unit AT.	1 c.cm. contains less than $^{1}/_{42}$ unit AT.
11	G.K.	4	Now Schick negative. 1 c.cm. contains more than $^{2}/_{5}$ unit AT.	1 c.cm. contains between 1 and 2 units AT.
12	G.L.	4½	Now slight positive Schick reaction. 1 c.cm. contains less than $^{1}/_{40}$ unit AT.	1 c.cm. contains less than $^{1}/_{42}$ unit AT.

10, 11 and 12 received immunizing injections of 2, 2 and 4 minims of toxin-antitoxin on November, 10, November 25 and December 1, 1927. The sera were obtained on February 27, 1928.

TABLE IV.

PROTECTION EXPERIMENTS WITH SERA FROM SCHICK POSITIVE AND SCHICK NEGATIVE CHILDREN.

No. of Serum.	Dose of Serum mixed with 0·01 c.cm. Toxin for Injection into Guinea Pigs of 250 grammes.	Result.
1	0·4 c.cm.	No infiltration; steady increase of weight.
	0·2 c.cm.	No infiltration; steady increase of weight.
	0·1 c.cm.	No infiltration; no loss of weight.
	0·05 c.cm.	Infiltration, necrosis; body weight not regained by fifteenth day.
	0·04 c.cm.	Died tenth day.
	0·02 c.cm.	Died seventh day.
2	0·7 c.cm.	Marked infiltration; weight not regained by eighteenth day.
3	0·5 c.cm.	Definite infiltration; no loss of weight.
	0·4 c.cm.	Definite infiltration; no loss of weight.
	0·2 c.cm.	Died fifth day.
4	0·4 c.cm.	No infiltration; no loss of weight.
	0·2 c.cm.	No infiltration; weight regained by fifth day.
	0·1 c.cm.	Slight infiltration; weight regained by fifth day.
	0·05 c.cm.	Infiltration; weight regained by eleventh day.
	0·04 c.cm.	Died fifth day.
	0·02 c.cm.	Died fifth day.

TABLE V.

DETAIL OF PROTECTION EXPERIMENTS WITH SERA OF CHILDREN WHO SURVIVED INJECTIONS ON JANUARY 27, 1928.

Initials and Case No.	Dose of Serum mixed with 0·01 c.cm. Toxin for Injection in Guinea Pigs of 250 grammes.	Result.
B.D. (Case 17)	0·7 c.cm.	Died in less than 63 hours.
E.D. (Case 21)	0·7 c.cm.	Died between 72 and 87 hours.
J.S. (Case 7)	0·2 c.cm.	Marked infiltration, necrosis; regained weight by fourteenth day.
	0·15 c.cm.	Marked infiltration, necrosis; regained weight by fourteenth day.
	0·1 c.cm.	Definite infiltration; not back to original body weight by fourteenth day.
W.S. (Case 6)	0·3 c.cm.	Only slight infiltration; no loss of weight; survived.
	0·2 c.cm.	Definite infiltration; regained weight by eighth day.
	0·1 c.cm.	Died between 72 and 87 hours.
B.P. (Case 13)	0·4 c.cm.	Slight infiltration; steady gain in weight.
	0·35 c.cm. (2 animals)	Infiltration; no definite loss of weight.
	0·2 c.cm.	Infiltration, necrosis; loss of weight; died eighth day.
	0·1 c.cm.	Died ninth day.
V.T.C. (Case 10)	0·7 c.cm.	Died between 72 and 87 hours.
F.B. (Case 2)	0·03 c.cm.	No infiltration; steady gain of weight.
	0·02 c.cm.	Infiltration, necrosis; regained weight by fourteenth day; survived.
	0·01 c.cm.	Infiltration, necrosis; loss of weight; died fourteenth day.
	0·006 c.cm.	Died third day.
	0·004 c.cm.	Died fourth day.
N.W. (Case 9)	0·006 c.cm.	Infiltration; no loss of weight.
	0·005 c.cm.	Infiltration, necrosis; regained weight by eighth day; survived.
	0·004 c.cm.	Infiltration, necrosis; regaining weight fifteenth day; survived.
	0·003 c.cm.	Died fourth day.
	0·0025 c.cm.	Infiltration; steady loss of weight; moribund eighteenth day.
	0·002 c.cm.	Died eighth day.

It will be noted that in two of the three sera of children in the first group who had no symptoms there was a particularly high content of antitoxin. The fourth child, C.W., who had no symptoms and from whom we failed to obtain blood, had had diphtheria thirteen months before. Her sister, N.W., who had the highest observed titre of antitoxin, was also said by her parents to have had diphtheria. These high titres of antitoxin were observed too soon after the last injection to have resulted from active immunization in Schick-positive individuals. The children probably had a high titre of antitoxin before January 27, the injection acting as a secondary stimulus. It may be argued that protection against diphtheria toxin was the cause of their freedom from symptoms. The absence of symptoms in V.T.C. cannot be explained in this way.

With regard to the children in the second and third groups, it is not possible to infer from the antitoxin content of their sera on February 15 whether they were Schick-positive or Schick-negative before January 27. In the second group the effect of the intramuscular injections of 4,000 units of antitoxin given while in hospital is still evident in the results of these titrations and it is possible that had they not had antitoxin the content of their sera in antitoxin would have been similar to that of those in the third group.

On the whole, these results are unfavourable to the view that free diphtheria toxin played any active rôle in causing the Bundaberg deaths.

APPENDIX 29.

The Effect of the Subcutaneous Injection of Diphtheria Toxin Together with the Bundaberg Staphylococcus.

Although we have been unable to dissociate toxin from toxin-antitoxin mixture by growing organisms in it, we thought it advisable to make a few experiments to ascertain whether the resistance of animals could be so reduced by diphtheria toxin that rapid death would result from the

injection of the Bundaberg organism. We have used only subcutaneous injections of both toxin and staphylococci in mice, guinea pigs and rabbits, injecting both in the same region. The staphylococci have been injected in saline emulsion made from eighteen to twenty-four hours old agar slope cultures and estimated by opacity.

In mice we did not determine the C.L.D. of the toxin, but injected in a dose of 0·1 c.cm. it failed to cause any symptoms. Injection of 100 million staphylococci was also without effect both alone and together with 0·1 c.cm. of diptheria toxin.

In guinea pigs the C.L.D. of the toxin was 0·01 c.cm. (Appendix 28) for animals of 250 grammes weight. The injection of 1,000 million staphylococci produces only a local lesion (Appendix 23). In four animals 1,000 million organisms were injected together with 0·002 c.cm. of diphtheria toxin and all survived. Of four animals injected with the same dose of staphylococci and 0·005 c.cm. of toxin, one died on the fourth day and the remainder and the control guinea pigs, which received only 0·005 c.cm. of toxin, survived.

The injection of 200 million staphylococci does not appear to shorten appreciably the time of death after administration of the C.L.D. of toxin. The average death time in ten animals which received 0·01 c.cm. of toxin was fifty-five hours and in four guinea pigs which received in addition this dose of staphylococci the average was fifty-two hours.

In rabbits of about one kilogram the C.L.D. was not accurately determined but was probably between 0·005 c.cm. and 0·01 c.cm. One-tenth of a cubic centimetre killed in twenty-seven hours, 0·05 in forty-one hours, 0·025 in fifty hours and 0·01 in eighty-six hours, while with 0·005 c.cm. two of three animals died on the fourth and seventh days. Rabbits injected with 0·00025 c.cm. of toxin with and without 1,000 million staphylococci survived and two rabbits which received 0·005 c.cm. of toxin and the same dose of staphylococci, survived beyond the tenth day.

These experiments show clearly that even large doses of diphtheria toxin do not enable the staphylococcus to kill rapidly, when administered by the subcutaneous route in the doses which we have used. It cannot be inferred with certainty from these negative results that an effect of this kind cannot be produced in man, but our evidence makes it unlikely and experience of staphylococcal infection complicating clinical diphtheria is also unfavourable to this possibility.

APPENDIX 30.

On Generalized Staphylococcal Infection.

By the kind permission of Dr. Cecil Purser, Chairman of the Board of Directors, Dr. A. H. Tebbutt has examined for us the records of the Royal Prince Alfred Hospital, Sydney, in order to obtain some insight into the incidence of the mortality and clinical features of generalized staphylococcal infections. The group studied is classified under the heading "Septicæmia and Pyæmia (non-puerperal)." From the year 1910 to 1928 eighty cases have been so classified, but one was definitely a case of sapræmia and another a readmission, leaving seventy-eight cases; fifty-four of these have been recorded as septicæmia and twenty-four as septico-pyæmia. It must be remembered that the distinction is not always clear and in many cases the diagnosis has been made on clinical grounds only. It should be emphasized that many conditions other than puerperal (*e.g.* sub-acute bacterial endocarditis) in which cultures of pathogenic organisms can be obtained from the blood during life, are not included in this group. In only one of the cases studied were there vegetations on the valves of the heart.

Of the fifty-four cases of septicæmia, forty-five died and nine recovered giving a mortality rate of 83·3%. Of the twenty-four cases of septico-pyæmia fourteeen died and ten recovered, giving a mortality of 58·3%. The mortality for the whole series was 75·6%.

In twenty-seven cases cultures were made from the blood during life. In eleven *Staphylococcus aureus*, in six streptococci, in one a pneumococcus, in one a colon bacillus and in eight cases no organisms were cultivated. In two cases the pathologist recovered staphylococci (*aureus* and *albus* respectively) which were regarded by him as contaminations. These have not been included as positive cases. Cultures obtained during life from various lesions yielded confirmatory evidence in many cases.

Autopsies were carried out only on thirty-nine cases. Where cultures were made they have supported the diagnosis by blood culture during life and the same organisms have been obtained from the heart's blood, the splenic pulp, septic infarcts in the lung or miliary abscesses in the kidney.

In a few cases in which blood cultures were not made during life, cultures obtained at autopsy were available for the diagnosis of the infecting organisms. These are as follows: Streptococcus, three cases; *Staphylococcus aureus*, six cases; *Staphylococcus albus*, one case; pneumococcus, three cases; colon bacillus, two cases.

Adding these to those in which the organism was obtained by blood culture, we find that the infecting organism has been determined in thirty-four cases as follows:

Staphylococcus aureus	17 cases ..	+ 52·94%
Staphylococcus albus	1 case ..	
Streptococcus	9 cases ..	26·47%
Pneumococcus	4 cases ..	11·76%
Colon bacillus	3 cases ..	8·83%

These figures indicate the frequency of occurrence of staphylococci in this somewhat restricted group of general infections.

The mortality rate in the eleven cases in which *Staphylococcus aureus* was obtained by blood culture during life was 100%, whilst that in the remaining eight cases in which other organisms were recovered, was 50% and in the eight cases in which the blood culture proved sterile 62·5%.

A careful study of the cases in this series indicates the gravity of generalized infection by *Staphylococcus aureus* and engenders a very marked respect for it as an infective agent. It is regrettable that from the point of view of accurate diagnosis and prognosis blood cultures are not more frequently and promptly carried out in severe infective conditions.

The principal features of this series of eighteen generalized staphylococcal infections are briefly considered:

1. Age.—Seven out of eighteen patients were under the age of twenty. The cases are distributed in decades as follows:

First decade, two, aged 14 months and 3 years.
Second decade, five, aged 13, 14, 14, 14 and 17 years.
Third decade, two, aged 20 and 25 years.
Fourth decade, five, aged 33, 33, 37, 37 and 39 years.
Fifth decade, three, aged 42, 47 and 48 years.
Sixth decade, one, aged 55 years.

2. Sex.—There were fifteen males and three females. Apart from this small group, in the whole series the great majority are males. This predominance may be correlated with the greated incidence of septic wounds (trauma) and boils and carbuncles in the male sex.

3. Primary foci.—These were as follows:

Lesion.	Number of Cases.
Boils or pustules	5
Mumps and boils	1
Septic wounds, generally superficial and slight	4
Superficial abscesses	2
Deep abscesses (iliac)	1
Cellulitis of the scalp	1
Blepharitis	1
Septic throat	1
No primary focus found	2
	18

4. Onset.—The symptoms as a rule are related to a primary septic focus which had sometimes been neglected The illnesses begin in various ways, sometimes with headache or pains in the chest, abdomen or limbs, occasionally

with vomiting or biliousness, feverishness, flushing of the skin and thirst or profuse perspiration. There was a definite chill in only one case and rigors in none. The local lesion sometimes flared up but in some cases only a serous exudate was found in the inflamed tissues. Enlargement of lymph glands is sometimes mentioned. Osteomyelitis or periostitis is noted in a few cases.

5. Temperature.—This is usually high but remitting one, two or three degrees daily. Temperatures of 104° F. and 105° F. are reached in most cases and in many there is a marked rise and in a smaller number a fall before death.

6. The Pulse.—The pulse is usually very rapid and soft. In most cases it varies daily from 120 to 160, but usually reaches 180 towards the end, even in adults.

7. Respiration.—This is always moderately rapid, varying daily from 25 to 40 on the average, but in several cases the rate is much higher, from 40 to 60. It tends to increase towards the end. The rate is apparently influenced by the extent of the pulmonary lesions, and fibrinous or septic pleurisy and pulmonary infarcts or abscesses are common. Respiration may be either rapid and quiet, grunting or gasping. Dyspnœa is occasionally a feature.

8. The Blood.—The illnesses were often of short duration or the patients were admitted in a very grave condition so that blood cultures were usually done rather late in the disease. In twelve cases the diagnosis was made by blood culture. In no case was the finding of a sterile blood followed by the autopsy finding of staphylococcal septicæmia or septico-pyæmia. The number of days between positive blood culture and death was as follows:

The day of death	3 cases
One day before death	3 cases
Two days before death	3 cases
Three days before death	1 case
Fourteen days before death	1 case
Fifteen days before death	1 case

Leucocyte counts were done in nine cases and showed considerable variations. In two of the cases there were only 8,000 leucocytes. Most commonly the number was between 10,000 and 20,000 (five cases). In the remaining two cases there were respectively 22,000 and 58,000. In nearly all the cases the count was made within a day or two of death.

9. The Skin and Connective Tissues.—In many cases no observations are recorded. In several there is profuse sweating which is sometimes an early symptom. The skin may be flushed, hot and dry. Apparently until near the end the colour may be good though pallor may be present. There may be marked lividity *post mortem*. In one case, a boy of fifteen years, two days before death the skin became ashy grey with blue lips, ears and nose and glazed eyes. In another case cyanosis was marked. A notable feature in a number of cases is the occurrence of small and large bullæ on the same limb as the primary focus, or fleeting red patches with œdema particularly on the forearms even though the primary focus be elsewhere. These are apparently metastatic lesions. In some there are secondary subcutanous abscesses. Secondary infection of joints is distinctly rare as compared with streptococcal and pneumococcal infections. Sometimes there is direct spread of a furuncle into adjacent subcutaneous and even muscular tissues.

10. Digestive System.—Troublesome symptoms are uncommon. The mouth is sometimes very dry, the tongue usually furred, the breath sometimes offensive. Vomiting if present is usually an early symptom. Diarrhœa is rare. The abdomen is often distended.

11. Excretory System.—Albuminuria is noted in several cases, glycosuria in only one case. Œdema of the limbs is always either inflammatory or follows venous thrombosis.

12. Nervous System.—Nervous symptoms are often prominent. Delirium is specially noted in seven cases. It is either very early or does not occur until the last two or three days. Not infrequently it gives place to coma towards the end. In another case there were headache and drowsiness deepening to coma. In two others also coma developed apparently without preceding delirium. In another case there was mental confusion. Marked restlessness sometimes accompanies the delirium. A young man of twenty-five years, on the day of his death "stared wildly with almost fixed pupils, clutching fiercely at objects or bystanders, then relapsed into general convulsive movements or twitchings." Pain is not infrequently noted early in the illness. Stiffness and pain about the local lesion, or pains in the limbs or joints are not infrequent. Pains in the chest are not uncommon and appear to be due to pleurisy or pleuro-pericarditis. Incontinence of urine and fæces is often coincident with states of delirium and coma. The outstanding features are the effects upon the higher centres—stupor, delirium or unconsciousness. This is in strong contrast with the lucid mental condition often seen in streptococcal septicæmia.

13. Special Features.—A pleural friction rub or an effusion from which staphylococci were cultivated is not uncommon. Lobar or bronchi-pneumonia has been suspected where congestion, infarction or abscess formation has been found at autopsy. Typhoid fever has been suspected but the Widal reaction has been absent when sought for.

14. Duration.—This is not always easy to determine. There is evidence that a decided rise in temperature and pulse rate and varying effects upon other systems mark the transition from local infection to septicæmia or septico-pyæmia. There is great variation in the length of the illnesses. One case had a raised temperature for only two days before death occurred. In other cases the duration was apparently three days, six days (3), seven, ten, eleven and about twenty-one days (3). Several patients died within twenty-four hours of admission to hospital but had been ill for a few days in their homes. The patient whose acute illness lasted only two days had nevertheless septic infarcts in the lungs and a few small abscesses in the kidneys.

15. Lesions at Autopsy.—There is no definite evidence that any patient with staphylococcal septicæmia or septico-pyæmia recovered. All the patients in whom the diagnosis was established by blood culture (12 cases) died and nine of them came to autopsy. These and the remaining six cases in which the diagnosis was made at autopsy make a total of fifteen autopsies. There is no significant difference between the findings in those cases which gave a positive blood culture and those in which blood cultures were not done.

The heart frequently shows some dilatation, particularly of the right side and not infrequently there is greyish or white clot in the right auricle and ventricle. In only one case is ulcerative endocarditis noted and that on the tricuspid valve with ulceration spreading through the adjacent heart wall and finally rupture and hæmopericardium. In one other case there was some mitral stenosis, but it was not thought that there was any acute endocarditis. The infarction of the lungs, therefore, is not due to malignant valvular disease. In only two cases is pericarditis recorded.

Lungs.—In most cases there are obvious pulmonary lesions. Fibrinous or septic pleurisy is frequently mentioned. Septic infarction is the most common lesion of the lung itself. The infarcts often stand out prominently and apparently progress rather rapidly to abscess formation. Large branches of the pulmonary artery supplying an extensive infarcted portion of one lobe were found definitely thrombosed in one case. In other cases there is congestion of the lung or collapse associated with a large pleural exudate. Subpleural and interstitial hæmorrhages may also occur. Septic mediastinitis is noted in one case.

Spleen.—Almost invariably the spleen is moderately enlarged and very soft, even diffluent. Sometimes it is noted as enlarged and friable. Hæmorrhages are also described but in no case infarcts or abscesses formation.

Liver.—This organ is usually described as enlarged and congested, sometimes also as fatty. Abscesses are not noted in any case.

Kidneys.—These are generally described as swollen or congested. Multiple abscesses are common, though in

several cases there were only a few abscesses. They are generally described as small or miliary. Infarction is described in two cases and hæmorrhages in two.

Alimentary Tract.—Lesions of note are rarely found, though the examination is often incomplete. In one case hæmorrhages into the wall of the small and large bowel are described.

Brain.—In several cases congestion of the external blood vessels is noted and in one case numerous sub-arachnoid hæmorrhages. In a number of cases it was not examined.

Skin.—In two cases there is special mention of marked lividity of the face and chest. In one it is noted that lividity was not confined to the dependent parts. In another there were abscesses and petechiæ in the skin of the back and in another blebs in the skin of the forearm.

The Primary Focus.—In two cases dissection of the veins from the lesion showed definite thrombosis.

Post Mortem Cultures.—The heart blood and spleen give possible findings, though in by no means all the cases were cultures made, since the blood had given staphylococci during life. Abscesses of the lung also gave staphylococci. Cultures from the brain were made in one case and gave positive results.

Summarizing these findings one may classify the autopsy findings and divide them into (i) septicæmia in the strict sense, without secondary foci, three cases; (ii) septico-pyæmia, with infarctions, abscess formations, septic inflammations of serous membranes, twelve cases.

Cryptogenic Cases.

These are rare and constitute an interesting type. There are two in this series.

One patient, a boy of fifteen years, had been feeling "off colour" for a few days, then complained of fleeting pains in the joints and later on of pains in the chest, especially during deep inspiration. There was some dyspnœa. No primary focus was found. On admission to hospital his temperature was 104° F., respiration 35-40, pulse 130. His skin was ashy grey and his lips, ears and nose bluish. A pleural friction rub was heard over the left base and crepitations over the right base. He became delirious, showed albuminuria and gave a positive blood culture. Diagnosis was not much helped by the leucocyte count in this case. Three days before death it was 10,000 with 79% neutrophiles and 4% eosinophiles. It cannot be too greatly stressed that leucocyte counts should be often repeated to bring out their real value in diagnosis and prognosis. Temperature, pulse and respiration rate rose steadily, reaching respectively 106·8° F., 190 and 80 on the day of death, five days after admission. There was no autopsy.

The second patient was a man of thirty-three years, who had a bilious attack with vomiting. On the following day he felt better. On the third day he felt sick again and had abdominal pains without diarrhœa. There were headache, anorexia, feverishness and some cough. On admission the temperature was 102° F., pulse rate 110 and respiration rate 40. The provisional diagnosis was typhoid fever, but the Widal test proved negative. The spleen was thought to be enlarged. There was a previous history of rheumatic fever, nineteen years before and a systolic murmur was detected. Nothing else was elicited in the physical examination. His temperature remained high—103° F. to 105° F.—the pulse only 110, but rising to 180 before death which occurred five days after admission. On the third day staphylococci were cultivated from the blood. The urine contained much albumin. At autopsy the skin showed much lividity. The bases of both lungs were greatly congested. The mitral valve showed some stenosis and there were a few vegetations, which were noted as "not recent," on the fimbriæ of the valve cusps. The left ventricle showed hypertrophy and dilatation. The spleen was much enlarged, friable and congested. The liver was large and fatty. Both kidneys showed cloudy swelling, petechial hæmorrhages and one showed two septic infarcts. There were hæmorrhages into the bowel wall, more notably in the colon. These hæmorrhages were "not infiltrated." Culture from the spleen and heart blood gave *Staphylococcus aureus*. No primary focus was found.

British Medical Association News.

SCIENTIFIC.

A MEETING OF THE NEW SOUTH WALES BRANCH OF THE BRITISH MEDICAL ASSOCIATION was held at the Glenfield Special School on May 10, 1928.

The members of the Branch were received by the Superintendent, Dr. Gilbert Phillips, and by Dr. Harvey Sutton. The routine of the school was explained and the children were seen at work in their several classes. On the wall of each room there was found a list of the children, with their ages, mental ages and intelligence quotient. Subsequently the children gave an exhibition of marching to music and of class singing.

The School Treatment of Mental Defectives.

DR. G. E. PHILLIPS, the Superintendent of the School, said that it was called the "Special School" probably for lack of a better name. It was special, however, in that it catered for a special type of child, had a special curriculum, special methods and a specially trained staff. This name had been selected rather than the name "Mental Defective School" or "School for the Feeble Minded," because the latter would not exactly describe all the types of children educated there and both had a popular connotation which would have the effect of excluding those children best able to profit by special instruction.

It had arisen because of a special educational need and the institution was to be for this reason regarded as a school with a home attached rather than as a home with a school attached. In other words, emphasis was on the educational rather than on the institutional aspects of the enterprise.

In all large schools there was a percentage of children who for various reasons, mainly low intellectual capacity, could not adequately profit from the curriculum and methods of instruction of the ordinary school and it was from these that the school recruited its pupils. Between the child suitable for the ordinary school and a child suitable for retention in an institution, such as the Newcastle Mental Hospital for example, there was a wide range of intellectual inferiority. It was from the superior end of this range that the Special School tried to recruit its pupils, leaving a large number whose needs were not catered for at all.

In the selection of pupils, no one was refused admittance if he was or would possibly in the future be capable of learning to read and write under the special conditions obtained there. In every case the child was given the benefit of the doubt and taken on trial, until experience resolved the doubt.

This resulted in the pupils exhibiting a wide range of intellectual ability, for although the great majority was of decidedly inferior intellectual development, some suffered from a specific inferiority which limited their power to learn and many were emotionally either retarded or abnormal. Several were also retarded intellectually and educationally through physical defects such as poor hearing.

Pupils were recruited mainly through reports from the school medical officers. They supplied details of children met with in their medical inspection of schools who were distinctly below normal in intelligence and whose schooling was thereby affected. The number of such children in public schools so reported amounted to between 1% and 1·5% of the school population or in round numbers between 3,000 and 4,500 individuals.

The parents of the most suitable of these children were circularized, the advantages of such a school as the Glenfield Special School were pointed out to them and they were invited to apply for admission for their children. Applicants were then examined by the Superintendent and, if deemed suitable, admitted. In the case of country applicants, admission was governed largely by reports from the school which the child attended, supplemented by examination when the child entered school.

Many children also came from other sources than the public schools. The Children's Court furnished a few,

mainly subnormal boys who had become truants through being misfits at school. Medical practitioners also supplied a number of pupils who had been brought to them for professional advice.

In opening a new school it was not always possible to obtain the exact type of material most suitable and this was noticeable particularly in the age range of the pupils. Now that the school was in full swing, however, the ages of accepted pupils were fixed between the limits of eight years and twelve years. Backward children of under eight years were not sufficiently developed to begin their academic education and, on the other hand, children who entered after twelve years of age, usually did not stay long enough to make their attendance worth while.

In an educational system special schools were necessary in the interests no less of normal children than of the backward children themselves. The presence of mentally undeveloped pupils in a class of normal children was an additional strain for the already sufficiently burdened teacher and for the sake of the great body of the normal children it was highly desirable to segregate educationally those who could not progress at somewhat near the normal pace. This desirable end could be achieved by having special classes in all large schools, by special day schools to which children might come from neighbouring schools, and, as in the case of the Glenfield School, by special residential schools where the training of the school pupil could be supplemented by special home training as well.

The aims of the school were twofold. As an individual the child had a right to such happiness as it was possible for him to obtain and to be provided with the means to obtain happiness. One source of happiness was through intercourse with his fellows, by means of conversation or the printed page. The school tried to achieve this aim in its teaching of elementary school subjects.

From the social viewpoint he had the right also to be so trained as to render him able to associate pleasantly with others and to be provided, if possible, with the means of gaining a livelihood. Of course this was the State's justification for spending public funds in special education. The school tried by special curriculum and special methods and with specially trained teachers and nurses to achieve these two aims, to develope the children as individuals for their individual happiness and also to lay the foundation for the earning of a livelihood later on. As the children became older, the second aim would be catered for more directly and the school would develope a vocational bias. At the present time with their older boys they were giving practice in simple farming operations and in gardening and with their older girls in household arts and management. As a preliminary to this the school gave a large amount of time to hand work of various kinds. But in what particular direction the school would develope could be determined only by experience.

DR. HARVEY SUTTON spoke about certain medical aspects of the work at Glenfield. The Special School had attached to it a medical officer, Dr. Alice McCloy and a close study was being made of types and regular health supervision was maintained. The types submitted at the meeting were intended to illustrate certain points. The first was that personal appearance and even expression were very unsafe guides in the diagnosis either for presence or absence of mental defect. Definite tests were necessary together with a record of the child's reaction in the school, in the home and at play, as well as the life history of its development, both physical and mental, especially as compared with a normal child. Dr. Sutton went on to say that certain special children had been submitted for clinical interest, although they formed the minority of the children dealt with. He was struck by the continual cropping up of disability occurring after meningitis in previously normal children. These tended to remain physically normal, but their mental development was seriously damaged. An occasional case of *encephalitis lethargica* was seen and rarely cases of true insanity in which progressive deterioration in a fairly normal child occurred.

He drew attention particularly to the work of the nurses in charge of the dormitories who represented the home side. The majority was fully trained and registered nurses: they were considered by the Department as part of the educational staff, their duties being the teaching of good habits, the first and most important item in the training of the mental defective. Not only did this include cleanliness and tidiness of every description, teeth, hair, skin, nails, clothes, beds, lockers *et cetera*, but every effort was directed to prevent bad habits to which these children were prone. This work had been particularly successful, especially in the control of constipation. A considerable number of children had been receiving purgatives at home and had contracted the habit. The regular exercise which the school provided had improved their muscle tone, a definite ritual of regular attendance irrespective of desire was insisted on and a diet modified to suit the circumstances. After a few months one child only was occasionally given an "Alophen" pill and since an apple was given at bedtime, this child had ceased to require the aid of drugs. A large supply of castor oil which had been originally placed in the dispensary remained practically intact. The work had thus demonstrated that the continuous and frequent use of purgatives so common in institutions dealing with children represented a failure to produce hygienic habits in children; this was possible on ordinary common sense lines.

The dormitory buildings were worthy of attention as representing the result of conferences with representatives of the mental hospitals together with the valuable advice of the late Eric Sinclair and Dr. Ralph Noble and others which had been put into practical shape by Colonel Wells, then Architect to the Department. The plans differed considerably from the American idea of a compact two-storey building with central heating. These were double dormitories, more on the pavilion plan, with cross ventilation of a natural type, each dormitory accommodated sixteen beds, with an allowance of eighty square feet per bed. There was a complete absence of any trace of odours so commonly found in institutions for children. This was due to the arrangement of one-way traffic by which the children entered through a bathing block where clothes and boots were discarded; the children then passed through a hot shower and got into their indoor dresses. Lavatories were also provided in the bathing block. These were placed at each end of the building, the central portion being devoted to an indoor playroom, particularly for evening use or in wet weather. Behind the playroom were the quarters for the two nurses, separate bedrooms, combined sitting room and kitchenette, together with linen room and store room. Ample veranda space was provided and the buildings were orientated so as to lie pointing north-east or north-west, the chief lighting wall lying facing eastwards and the veranda westwards. The prevailing winds for bad weather were on the south and west and the hot afternoon sun from the west was trying in summer. Eventually windbreaks of trees would considerably improve the comfort of the place. Water sewerage was provided, together with electric light and a plentiful hot water supply. All meals, except for sick children, were provided in the central dining room. The main block of buildings included school, dining room and laundry, with Matron's quarters and staff dining rooms. Four cottages accommodated one hundred and twenty-eight children and a small isolation block of four beds was also provided. The land was classed as good second class land and was as good as any land within the same distance, twenty-five miles, of Sydney. The kitchen garden, run by the boys, was already doing well. Eventually, as the boys grew older (very few exceeded the age of fourteen) the outdoor farm and orchard work would form an important item in their training.

Dr. Sutton felt the members of the medical profession could do a great deal more for the future progress of the school by their helpful criticism of the arrangements of the Special School. As far as the school itself was concerned, work had already reached a high pitch of efficiency under Miss Pfitzner. As the institution grew, the homes would provide more and more satisfactory classification, the chief difficulty being the tendency for lower grade children to apply for admission in a far higher proportion than those of higher grades. One of the essential ideas for the creation of this school was to provide a training ground for teachers and a greater experience of the problem of the feeble-minded. It was not fair, especially at this early stage of development, to burden the school

with children requiring actual nursing or of such low grade that they could not benefit to any considerable extent from educational effort in school. The lower the grade, the more obvious the condition and although the institution could be filled several times over with low grade children, those for whom most could be done were the high grade feeble-minded, those who deviated least from normal. These, though unsuitable for the ordinary school because of their unfavourable reaction to the conditions which suited normal children, yet were unsuitable for a special school and, indeed, did best under conditions of educational life specialized to their needs. Parental pride or wilful blindness made it difficult to obtain the consent of the parents to the admission of their children to a school at which they could see children with low grade mental defect. They were trying more and more to reduce the proportion of low grade children and to concentrate on the high grade types. The support of the profession would be greatly welcomed in dealing with high grade defectives. If a doctor in practice would support the finding of the school medical officers and educational psychologist as to the need of such children going to a special school, it would smooth out many difficulties and greatly assist the welfare of this much misunderstood type of child.

So far little medical treatment has been attempted of the causes of mental defect. They were still searching for satisfactory indications for use, for example, of endocrines; although the probability was that in the future some at least of these children would respond to such treatment as affecting growth both of mind and body; so far the indications were on the whole too unreliable as a basis for treatment.

NOMINATIONS AND ELECTIONS.

THE undermentioned have been elected members of the South Australian Branch of the British Medical Association:

Trundinger, Ronald, M.B., B.S., 1909 (Univ. Adelaide), Adelaide.

Solomon, George Herbert, M.B., B.S., 1927 (Univ. Adelaide), Adelaide.

THE undermentioned have been nominated for election as members of the New South Wales Branch of the British Medical Association:

Lumley, George Frederick, M.B., Ch.M., 1926 (Univ. Sydney), 70, Bestic Street, Rockdale.

Stevens, Stanley Gordon, M.B., B.S., 1928 (Univ. Sydney), Mental Hospital, Orange.

Obituary.

JOHN DANIEL KING SCOTT.

DR. JOHN DANIEL KING SCOTT passed away suddenly on May 28, 1928, at his residence, Churston, Finch Street, East Malvern, aged fifty-seven years. He was the youngest son of the late Robert Scott, grazier, of Mount Buninyong, near Ballarat, Victoria. Having begun his school education at Scotch College, Melbourne, in 1876, he matriculated in 1886 and entered Ormond College, Melbourne University, in 1889, graduating as Bachelor of Medicine and of Surgery in 1896. He took an active part in the life of his college and was a member of the Students' Committee. After graduation he was appointed Resident Medical Officer at the Melbourne Hospital for six months and later served in the same capacity for about a year at the Women's Hospital. After taking a trip to Japan as a ship's Medical Officer, he commenced practice in Lygon Street, Carlton, Victoria.

In December, 1899, he married Isabelle Milne Garlick, daughter of the late Reverend John Garlick, of Bendigo. He transferred his practice to Queenscliff in 1900 and stayed there till 1907. Here he took an active part in civic and military life. He was a member of the Shire Council from 1904 to 1907 and was also Health Officer and Medical Officer to the Garrison Artillery. In 1907 he bought Doctor Alex Reid's practice in Sydney Road, Brunswick, but found that the lodge work was too much for his health. In 1912 he sold his Brunswick practice and later moved to East Malvern which he worked till the time of his death. He was on the staff of the Children's Hospital, Carlton, as Honorary Physician to Out-patients until 1915. During the war he served as a recruiting medical officer in Melbourne and as instructor to Red Cross workers. He was anæsthetist at Number 5 Australian General Hospital and at the Caulfield Military Hospital with the rank of captain and also served as medical officer at Port Phillip Heads during war time.

John Daniel King Scott was a keen gardener, golfer and bowler and excelled at the latter game. In the last few years of his life his sport and later his work were interfered with by rheumatism and by the effects of an injury incurred when horse jumping as a young man.

He was an active member of the East Malvern Presbyterian Church, serving on the Board of Management and as a member of the Session. He was a member of the Council of the Old Scotch Collegians' Association and of the Committee of the Old Ormond Students' Association and of the Ormond College Council up to the time of his death.

He served for some time as a member of Council of the Victorian Branch of the British Medical Association. Thus he lived a full and valuable life, giving of his best in the kind of service to which he was peculiarly suited, loved and trusted by his patients and his friends. He leaves three sons and a daughter.

While practising at Carlton and in later years Doctor King Scott was closely associated with Doctor Frank Hobill Cole, who writes:

> Outstanding qualities in the personality of Dr. J. D. King Scott were loyalty and courage. He was intensely loyal to his family, to his old schools (Scotch College and Ormond College), to his friends, professional and other, to his church and to his country.
>
> His courage in long and painful disabilities was very real and until the loss of his devoted wife some eighteen months ago, no diminution of his cheerfulness was ever observed, but in that great sorrow the light of life dimmed and the zest faded. Still his interest in his old school never slackened and he rarely missed a cricket or football match or a boat race. He had an abounding faith in young folk and inspired them with lofty ideals.
>
> A leal comrade, a wise and courteous physician has gone from among us and many there are who will miss the genial smile, the wide sympathy, the encouraging word and the helping hand of Dr. J. D. King Scott.

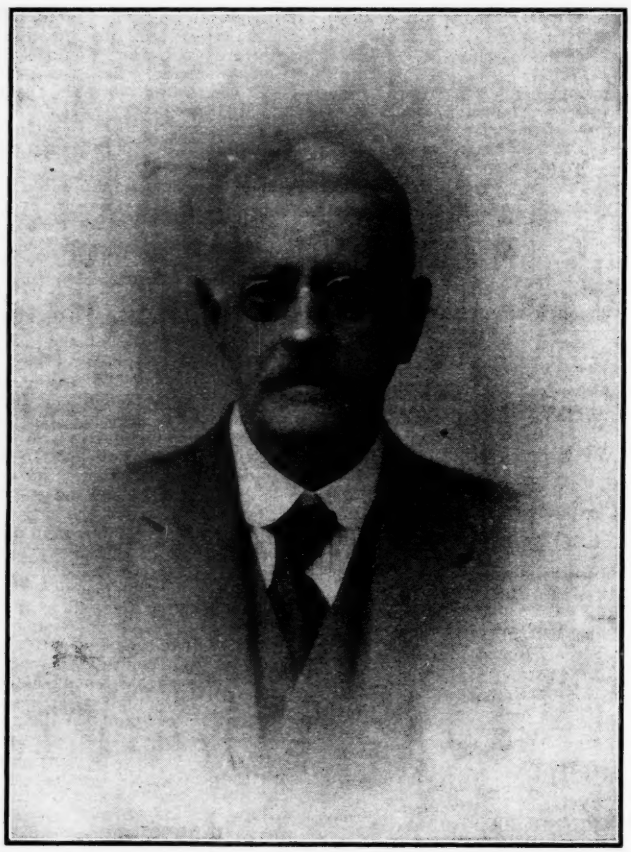
THOMAS MORGAN MARTIN.

ON May 25, 1928, Thomas Morgan Martin, one of the pioneers of the Australian Army Medical Corps, patriot, doctor and gentleman, passed to "the other side" to the great sorrow of his many friends, patients and contemporaries. His last illness was not of long duration. Quite recently his colleagues at the annual dinner of the Returned Medical Officers' Association expressed their profound regret that Colonel Martin had been prevented from attending on account of indisposition. He had accepted the invitation of the South African Soldiers' Association to be the guest of honour at a dinner on May 25, but forty-eight hours before he was no longer among the living.

Thomas Morgan Martin was born in Dublin in 1854. He was the son of John W. Martin, a learned and distinguished man of letters. He attended North's School in Dublin and in due course he entered Trinity College. He took a degree in arts at the University of Dublin and then entered the medical school in Edinburgh. In the year 1879 he obtained the diplomas of Licentiate of the

Royal Colleges of Physicians and Surgeons. Shortly after he left the old world for Australia and became a registered medical practitioner in Sydney in the year 1885. The medical community of Sydney forty-two years ago was small and enterprising. Facilities for post-graduate study did not exist and each member of the profession had to rely on the knowledge he had acquired during his training at home and on the experience he had had in private and hospital practice. Those who succeeded, were perforce men of resource and self-reliance, men who were prepared to face the difficulties of their profession unaided and often with indifferent equipment. Thomas Morgan Martin succeeded in his practice and gained the esteem of his colleagues, the confidence and affection of his patients and the admiration of the community.

From an early age he manifested an intense desire to further the interests of the Empire. He joined the Army Medical Corps in New South Wales as Lieutenant in 1897. No one was surprised when, at the outbreak of the South African War, Martin joined the New South Wales Forces. In 1899 he served as medical officer of the field hospital of the Army Medical Corps of the New South Wales Forces. He saw much fighting, was present at many battles and contributed in no small measure to the high reputation gained by the medical corps of his colony. He distinguished himself on the field and his services were recognized in an official manner. He was awarded the Queen's Medal with six clasps. Later he was appointed secretary to the late William D. C. Williams, then Principal Medical Officer of the Mounted Division under General Ian Hamilton and afterwards under General Archibald Hunter. His close association with General Williams inspired him to throw his energy into the task of founding a permanent Army Medical Corps. Within a few years after Australia had attained the status of a commonwealth, this objective of a handful of strong pioneers working together with Williams was attained. It was the same coterie of untiring men who kept the nucleus of the Australian Army Medical Corps alive and efficient up to the time of the Great War. Had it not been for them Australia would have been found utterly unprepared for the great task awaiting the Australian Imperial Forces. During the South African War he was promoted to the rank of Captain and a little later he attained his majority. In 1906 he passed the necessary examinations and attained the rank of Lieutenant-Colonel and was then appointed Officer Commanding the First Field Ambulance.



In 1914 Thomas Morgan Martin left Australia on board the *Kyarra* in charge of the Second Australian General Hospital. He was at that time sixty years of age. His experience in military matters, his previous war record and his keenness more than counteracted the disadvantage attaching to his seniority. At first he was in command of the hospital at Mena and later he was transferred to Gezerah. He was promoted to the rank of full Colonel after his return to Australia. After the Gallipoli campaign had come to an end, he went to France and was placed in charge of the No. 2 Australian General Hospital at Wimereux. In 1916 he was created a Commander of the Order of Saint Michael and Saint George for his services during the Gallipoli campaign. Towards the end of the war he was given command of the stationary hospital in England at Southall. These are merely landmarks. It would need a small book to detail his activities at each stage of the pilgrimage from Australia to the various seats of war and his return to New South Wales. But even when he reentered general practice and took his wonted place in the civil community, he was not satisfied to leave the affairs of his country to take care of themselves. He had seen how a great medical service had to be built up during the war out of a mere nucleus. He had watched the training of immense numbers of young men and had taken pride in the achievements of the reconstituted Australian Army Medical Corps. He had learned that there was a grave danger that the medical profession might relapse into the same state of unpreparedness if those who had become versed in the routine of field activity and campaign requirements, failed to keep the old corps in a state of efficiency. He was totally unwilling to relinquish his hold on military matters and to the end his main endeavour was to maintain the advantage gained of experience in the years of war.

He was keenly interested in civil ambulance work and was a member of the New South Wales Ambulance Transport Board. In many other respects he brought his experience to bear on the safety and welfare of his countrymen. Among men of all classes he was deservedly popular. In his practice he sacrificed comfort for his patients' weal and spared himself not at all. His colleagues near and far recognized his sterling merits, his attention to duty and his many admirable qualities. His friends loved the kindly man and envied his loyalty and geniality. Deep sympathy is felt with his widow and his three sons and three daughters.

Correspondence.

ANATOMICAL ABNORMALITIES.

SIR: During the course of dissection of a cadaver recently I found some rather interesting variations from the normal morphology.

An interesting, though more common, deviation was the presence of eight costal cartilages all reaching the sternum. The first six of these adopted the usual articulation with the *corpus sterni*, as also the eighth. The seventh articulated with the last segment of the sternum in the same manner as the sixth cartilage.

A more unusual deviation was the arrangement of the cords of the brachial plexus. The fifth and sixth anterior cervical rami formed one trunk, while the seventh, eighth and first thoracic all joined to form one other trunk only. These formed by division into three cords which, however, all maintained a position lateral to the axillary artery. This meant that the ulnar nerve arose lateral to the artery and passed posteriorly to it to attain its medial position. Also the lateral and "medial" contributions of the cords to the median nerve both passed from the lateral side of the artery. But the most interesting deviation and the one that led me to write this note was the strange origin of the middle colic artery. At least some well known and authentic manuals of surgery recommend, in the course of a pylorectomy, that the surgeon should, if possible, tie the gastro-duodenal artery at once instead of ligating in turn the right gastro-epiploic and the superior pancreatico-duodenal arteries. If this had been done in the case of the cadaver in question, there would have been one more undiagnosed cause of death. For in this case the gastro-duodenal artery was as large as an adult coronary artery, left the hepatic at the usual place, ran down anterior to the neck of the pancreas and then, dividing into left and right branches, entered the transverse mesocolon to supply to the transverse colon. Just before entering the mesocolon it crossed anteriorly to the right colic artery which came off the superior mesenteric artery a little higher up than usual and turned at once towards the hepatic flexure.

Yours, etc.,

L. J. SHORTLAND.

DIATHERMY APPLIED TO MEDICINE.

SIR: Dr. Pern's interesting article should stir up more interest in diathermy. I cannot quite follow all his conclusions, but, as the pioneer of diathermy in Australia, I welcome his enthusiasm and hope many more will give diathermy a trial. I think it should be a part of every practitioner's armamentarium. It is certainly worth while in all resistant cases of asthma and chronic lung conditions. Many cases of piles, especially when external and inflamed, can avoid operation by its application Several I know of have remained free from trouble for several years. I consider that if operation be necessary, a local anæsthetic and needle coagulation is much to be preferred to the ordinary excision method. I have not had much success in treatment of inflamed prostate, though Cumberbatch is definite as to its value. It seems of value in some cases of resistant suppurative *otitis media*. I wonder how long it will be before surgeons wake up to the possibilities of Wyeth's endothermic knife. Removal of tonsils by "desiccation" should in my opinion be reserved for cases unsuitable for ordinary enucleation and though I have not done many enucleations with the endothermic knife, the patients have decidedly less pain.

In all cases, however, I would urge, especially in the group of "lumbago and sciatica," that along with diathermy every avenue should be explored to ascertain the root cause.

Yours, etc.,

W. KENT HUGHES.

22, Collins Street, Melbourne.
Undated.

THE CHAIR OF OBSTETRICS AND THE APPOINTMENT OF PROFESSORS.

SIR: In my official capacity as Dean of the Faculty of Medicine I cannot allow your leading articles of June 9 and June 30 to pass without correction, because neither of them represents the facts as regards this University.

Sir Edward Mitchell has very properly corrected the somewhat extraordinary misstatements of your former leading article and as I was very naturally in the confidence of Sir Edward Mitchell and his colleagues it is almost unnecessary to state that I know Sir Edward to be right and your article to be wrong. It was only at the last meeting of the Faculty of Medicine in this University that I categorically denied the rumours upon which your article was unfortunately based, and I did so with the documentary evidence in my possession.

As regards the second leading article on June 30, on "The Appointment of Professors," it is a pity it was ever written, as it misleads English applicants for Australian chairs. Our English advisory committees are specially instructed in the exact converse of what your article states and, so far as Melbourne is concerned, have nothing whatsoever to do with the selection of occupants for our chairs. Having made an entirely erroneous statement it is surely not over courteous to add that a procedure which does not exist "further implies that the faculty in the Australian university is incompetent to carry the responsibility." Personally, my own Faculty is entirely competent to deal with this and a good deal more than a university lay council seems disposed to recognize.

Your concluding claim that the Australian should not be placed at a disadvantage and your denouncement of an illogical system long since abandoned are admirable sentiments which Melbourne has recognized and acted upon for many years.

Yours, etc.,

RICHARD J. W. BERRY,
Dean of the Faculty of Medicine,
University of Melbourne.

June 3, 1928.

[We are indebted to both Sir Edward Mitchell and Professor Berry for information contained in their letters. In view of the importance of the subject of the training of students in obstetrics, it is proposed to await further correspondence before publishing a reply.—EDITOR.]

Post-Graduate Work.

POST-GRADUATE WORK IN MELBOURNE.

THE Melbourne Permanent Committee of Post-graduate Work announces that the usual annual refresher course will be held from August 6 to August 17, 1928. The fee for this course is three guineas.

The course of six lectures by Professor F. R. Fraser will be delivered at the Medical Society Hall, East Melbourne, at 8.30 p.m. on the dates given below.

Tuesday, August 7, 1928.—"Causes of Dyspnœa and the Clinical Types."
Wednesday, August 8, 1928.—"Cardiac Dyspnœa."
Friday, August 10, 1928.—"The Therapeutic Uses of Digitalis."
Monday, August 13, 1928.—"Diuresis."
Wednesday, August 15, 1928.—"Heart Failure in Graves's Disease."
Friday, August 17, 1928.—"Hæmatemesis and Its Management."

Members wishing to attend the course should lose no time in sending in their applications to the Joint Honorary Secretaries. The fee for the course is three guineas.

Books Received.

THE EVOLUTION OF MAN SCIENTIFICALLY DISPROVED IN FIFTY ARGUMENTS,, by Rev. William A. Williams, D.D.; 1928. New Jersey: William A. Williams. Crown 8vo., pp. 125. Price: $1.00 net.

THE ULTRA-VIOLET RAYS, by Arnold Lorand, M.D.; 1928. Philadelphia: F. A. Davis Company. Royal 8vo., pp. 258. Price: $2.50 net.

STUDIES IN THE PSYCHOLOGY OF SEX; Volume VII: Eonism and other supplementary studies, by Havelock Ellis; 1928. Philadelphia: F. A. Davis Company. Royal 8vo., pp. 539. Price: $5.00 net.

STATISTICAL METHODS FOR RESEARCH WORKERS, by R. A. Fisher, Sc.D.; Second Edition, Revised and Enlarged; 1928. Oliver and Boyd. Demy 8vo., pp. 280. Price: 15s. net.

THE PRACTICAL MEDICINE SERIES COMPRISING EIGHT VOLUMES ON THE YEAR'S PROGRESS IN MEDICINE AND SURGERY: Under the General Editorial Charge of Charles L. Mix, A.M., M.D.; Nervous and Mental Diseases; 1927. Chicago: The Year Book Publishers. Crown 8vo., pp. 387, with illustrations.

OUTLINES OF DENTAL SCIENCE: DENTAL ANATOMY, HUMAN AND COMPARATIVE, by David Headridge, L.D.S., R.C.S. (England), and S. Knight Gibson, L.D.S. (Man.); 1928. Edinburgh: E. and S. Livingstone. Crown 8vo., pp. 244, with illustrations. Price: 8s. 6d. net.

REQUISITES AND METHODS IN SURGERY FOR THE USE OF STUDENTS, HOUSE SURGEONS AND GENERAL PRACTITIONERS, by Charles W. Cathcart, C.B.E., M.A., M.B., C.M. (Ed.), F.R.C.S. (England and Edinburgh), and J. N. Jackson Hartley, O.B.E., M.B. (Ed.), F.R.C.S. (England and Edinburgh); 1928. Edinburgh: Oliver and Boyd. Crown 8vo., pp. 484, with illustrations. Price: 12s. 6d. net.

Diary for the Month.

JULY 16.—New South Wales Branch, B.M.A.: Organization and Science Committee.
JULY 17.—New South Wales Branch, B.M.A.: Executive and Finance Committee.
JULY 17.—Tasmanian Branch, B.M.A.: Council.
JULY 18.—Western Australian Branch, B.M.A.: Branch.
JULY 18.—Central Northern Medical Association, New South Wales.
JULY 24.—New South Wales Branch, B.M.A.: Medical Politics Committee.
JULY 25.—Victorian Branch, B.M.A.: Council.
JULY 26.—New South Wales Branch, B.M.A.: Branch.
JULY 26.—South Australian Branch, B.M.A.: Branch.
JULY 27.—Queensland Branch, B.M.A.: Council.

Medical Appointments.

Dr. Robin James Hester Spark (B.M.A.) has been appointed Government Medical Officer at Gatton, Queensland.

* * * *

Dr. F. W. Cotton (B.M.A.) has been appointed District Medical Officer and Public Vaccinator at Wyndham, Western Australia.

* * * *

Dr. Charles William Adey (B.M.A.), Dr. Kenneth Bertram Hope (B.M.A.), Dr. Henry Erskine Downes, Dr. Herbert Spencer Roberts (B.M.A.) and Dr. Raphael West Cilento (B.M.A.) have been appointed to act as Medical Inspectors of Seamen under the provisions of the *Navigation Act* 1912-1926.

* * * *

Dr. Marjorie Phyllis Casley Thomas has been appointed Acting Resident Medical Officer at the Adelaide Hospital, South Australia.

Medical Appointments Vacant, etc.

For announcements of medical appointments vacant, assistants, *locum tenentes* sought, etc., see "Advertiser," page xvi.

AUSTIN HOSPITAL FOR CHRONIC DISEASES, HEIDELBERG, VICTORIA: Junior Resident Medical Officer.

UNIVERSITY OF HONGKONG: Reader in Biology.

Medical Appointments: Important Notice.

MEDICAL practitioners are requested not to apply for any appointment referred to in the following table, without having first communicated with the Honorary Secretary of the Branch named in the first column, or with the Medical Secretary of the British Medical Association, Tavistock Square, London, W.C.1.

BRANCH.	APPOINTMENTS.
NEW SOUTH WALES: Honorary Secretary, 30 - 34, Elizabeth Street, Sydney.	Australian Natives' Association. Ashfield and District Friendly Societies' Dispensary. Balmain United Friendly Societies' Dispensary. Friendly Society Lodges at Casino. Leichhardt and Petersham Dispensary. Manchester United Oddfellows' Medical Institute, Elizabeth Street, Sydney. Marrickville United Friendly Societies' Dispensary. North Sydney United Friendly Societies. People's Prudential Benefit Society. Phœnix Mutual Provident Society.
VICTORIAN: Honorary Secretary, Medical Society Hall, East Melbourne.	All Institutes or Medical Dispensaries. Australian Prudential Association Proprietary, Limited. Mutual National Provident Club. National Provident Association. Hospital or other appointments outside Victoria.
QUEENSLAND: Honorary Secretary, B.M.A. Building, Adelaide Street, Brisbane.	Members accepting appointments as medical officers of country hospitals in Queensland are advised to submit a copy of their agreement to the Council before signing. Brisbane United Friendly Society Institute. Stannary Hills Hospital.
SOUTH AUSTRALIAN: Secretary, 207, North Terrace, Adelaide.	All Contract Practice Appointments in South Australia. Booleroo Centre Medical Club.
WESTERN AUSTRALIAN: Honorary Secretary, 65, Saint George's Terrace, Perth.	All Contract Practice Appointments in Western Australia.
NEW ZEALAND (WELLINGTON DIVISION): Honorary Secretary, Wellington.	Friendly Society Lodges, Wellington, New Zealand.

Medical practitioners are requested not to apply for appointments to positions at the Hobart General Hospital, Tasmania, without first having communicated with the Editor of THE MEDICAL JOURNAL OF AUSTRALIA, The Printing House, Seamer Street, Glebe, New South Wales.

Editorial Notices.

MANUSCRIPTS forwarded to the office of this journal cannot under any circumstances be returned. Original articles forwarded for publication are understood to be offered to THE MEDICAL JOURNAL OF AUSTRALIA alone, unless the contrary be stated.

All communications should be addressed to "The Editor," THE MEDICAL JOURNAL OF AUSTRALIA, The Printing House, Seamer Street, Glebe, Sydney. (Telephones: MW 2651-2.)

SUBSCRIPTION RATES.—Medical students and others not receiving THE MEDICAL JOURNAL OF AUSTRALIA in virtue of membership of the Branches of the British Medical Association in the Commonwealth can become subscribers to the journal by applying to the Manager or through the usual agents and booksellers. Subscriptions can commence at the beginning of any quarter and are renewable on December 31. The rates are £1 for Australia and £2 5s. abroad *per annum* payable in advance.